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TREATMENT OF SULFONAMIDE RESISTANT URINARY TRACT INFECTIONS WITH A COMBINATION OF SULFONAMIDE AND TRIMETHOPRIM

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Abstract. The aim of this study was to investigate the effect of treatment with combination of sulfamethoxazole and trimethoprim on patients with long-lasting urinary tract infections caused by bacterial strains resistant to sulfonamides. The series consisted of 55 geriatric patients with significant bacteriuria, which proved to be resistant to sulfonamide in vitro. The patients were divided into three groups. The 19 patients of the first group were treated with trimethoprim alone, the daily dose being 320 mg. The 24 patients of the second group were treated with combination of 1.6 g of sulfamethoxazole and 320 mg of trimethoprim (Trimesulfa®, Lääke Oy) given daily in two doses during 15 days. The 12 patients of the third group were treated with sulfamethoxazole, the daily dose being 1.6 g. The effects of the treatment in the three groups were as follows: significant bacteriuria disappeared in 21 out of 24 patients treated with sulfamethoxazole-trimethoprim, in 8 out of 19 patients treated with trimethoprim and in 1 out of 12 patients treated with sulfamethoxazole. It was concluded that combined treatment with sulfamethoxazole and trimethoprim is indicated in sulfonamide-resistant infections as well, and the treatment is significantly more effective than treatment with trimethoprim alone. The results give reason to assume that sulfonamide and trimethoprim have a synergistic effect in the treatment of urinary tract infections. The MIC values were lower for trimethoprim when the treatment was combined than when the treatment was carried out with trimethoprim alone. The differences were indicative but not significant. The serum folic acid concentration was practically unchanged after treatment with trimethoprim and with the combination of sulfamethoxazole and trimethoprim.

Combined treatment with sulfonamide and trimethoprim has proved to be effective in treatment of bacterial infections. This treatment has recently attained great interest among the clinicians. The most encouraging results have been reported in treatment of respiratory and urinary tract infections.

The first clinical experience with sulfonamide and trimethoprim in treatment of urinary tract infections was reported by Sourander and Werner in 1967 (4). The results of this study supported the opinion that the effect of sulfonamide and trimethoprim was synergistic. This synergism has also been confirmed in vitro. The most convincing recent study has been that by Grüneberg and Kolbe (1). In this study the effect of the combination of sulfamethoxazole and trimethoprim 1:5 was found very effective in infections caused even by sulfonamide-resistant bacterial strains.

Trimethoprim is a pyrimidine derivative. It is a folic acid antagonist which has been known for long and been used in the past in treatment of malaria. The effect of trimethoprim is based on its inhibitory effect on dihydrofolic acid reductase of microorganisms. This effect causes a failure of the purine synthesis. The inhibition of the dihydrofolic acid reductase by trimethoprim is only slight in man. Treatment with the combination of sulfonamide and trimethoprim acts at two different stages of the same biosynthesis in the microorganisms.

In treatment with sulfonamide and trimethoprim the choice of a particular sulfonamide depends on pharmacokinetic properties. Sulfamethoxazole has proved to be the most suitable sulfonamide, having an almost similar elimination rate to that of trimethoprim.

The aim of the present study was to investigate the effect of treatment with the combination of sulfonamide and trimethoprim on patients with long-lasting urinary tract infections caused by bacterial strains resistant to sulfonamides. The sulfonamide resistance has been proved in vitro

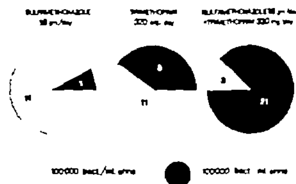


Fig. 1. Response to treatment with sulfamethoxazole and trimethoprim in 53 patients with significant bacteriuria. Duration of treatment 15 days.

by the disc method. Thus the series is primarily not suitable for treatment with sulfonamides alone. Treatment with the combination of sulfonamide and trimethoprim is only indicated when this therapy yields a better result than treatment with trimethoprim only

The problem is summarized as follows: is combined treatment with sulfonamide and trimethoprim also indicated in sulfonamide-resistant infections and is it more effective than treatment with trimethoprim alone?

MATERIAL AND METHODS

The series consisted of 55 patients, 5 omen and 3 men (mean age 73.6 years), from the Internal Medicine Department of the City Hospital of Turku, Finland. These patients had significant bacteriuria, and the bacteria were sulfonamide-resistant in vitro. All patients had been admitted to the hospital because of chronic diseases. They *all had been treated for urinary tract infection several* times. The earlier treatment consisted of administration of various antibiotics, sulfonamides or nitrofurantoin. The infection had lasted for years in most cases, but the renal function had remained practically unaffected in many of these aged patients. Out f 55 patients, the serum creatinine level exceeded 1.4 mg 100 ml in 11. Nine patients had serum creatinine value ranging from 1.5 to 1.8 mg/100 ml. The serum creatinine level was in one patient 2.2 mg/100 ml and in another 4.2 mg 100 ml. The specific weight of the morning urine was less than 1.015 in nine patients. The criterion for significant bacteriuria was more than 100 000 bacteria ml urine and the criterion for positive result of the treatment was the disappearance of the significant bacteriuria after the treatment.

Forty-three patients were divided into tw groups at random. The first group consisted of 19 patients treated with trimethoprim, the second group consisted of 4 patients treated with a combination f sulfamethoxazole and trimethoprim. The daily dose was 320 mg of trimethoprim and, when the combined treatment was given, 1.6 g of sulfamethoxazole and 320 mg of trimethoprim (Trimosulfa®). The duration of the treatment was 15 days. The drugs were administered twice a day—tw tablets before meals. The intake of tablets was supervised by the nurses of the ward. Bacterial culture of the urine and counting according to Kass (3) were performed before the treatment and three days after it.

T elve patients ere treated with sulfamethoxazole alone. The reason for including this group in the study was the demand for comparing the clinical results of the treatment to sulfonamide resistance in vitro. These patients were also chosen t random mong the patients in the hospital, so that they formed a group fully comparable with the other patient groups of the study

RESULTS

As expected, the effect of sulfonamide on these patients was negative with one exception. The result of the treatment with the combination of sulfonamide and trimethoprim was significantly better than that obtained with trimethoprim alone Out of 19 cases treated with trimethoprim alone eight were cured. When the patients were treated with the combination of sulfonamide and trimethoprim, 1 of .4 were cured (Fig. 1).

The effect of the combined treatment in infections caused by E. coli was as good as the result obtained in treating infections caused by Proteus. In infections caused by Pseudomonas there is no effect of trimethoprim, because of primary resistance. This series included one patient with an infection caused by Pseudomonas—the result of the treatment in this particular case was negative, as expected (Table I).

Estimation of the minimum inhibitory concentration (MIC) for sulfamethoxazole trimethoprim, and the combination of sulfamethoxazole and trimethoprim, was made by the disc dilution method. The broth was Oxoid Diagnostic Sensitivity Test Agar containing 5% of haemolyzed horse blood (2, 5). The MIC values were lower for trimethoprim and sulfamethoxazole than for trimethoprim alone. The average of the MIC values for trimethoprim was 0.41 ± 0.54, and 0.21 ± 0.14 when trimethoprim was combined with sulfamethoxazole. The differences of the mean values are not significant. This concerns the whole material as well as the coli and Proteus strains separately There seems to be a clear trend, however that the trimethoprim-MIC values for sulfamethoxazole and trimethoprim are

Table I. *Response to treatment evaluated by bacterial colony count*

BACTERIAL CULTURE	TREATMENT					
	SULFAMETHOXAZOLE		TRIMETHOPRIM		SULFAMETHOXAZOLE TRIMETHOPRIM	
BACT. in URINE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E. COLI						
PROTEUS MIRABILIS						
OTHER STRAINS						
TOTAL		[illegible]	[illegible]	[illegible]	[illegible]	

on a lower level than those for trimethoprim alone. Generally it can be stated that the trimethoprim-MIC values for the combined treatment are on a level where trimethoprim alone is not effective. This observation gives reason to assume that the effect of sulfonamide and trimethoprim is synergistic.

The serum folic acid concentration was practically unchanged after treatment with trimethoprim and with the combination of sulfamethoxazole and trimethoprim. The mean serum folic acid concentration was 4.1 ng/ml before and 4.6 ng/ml after the treatment. In none of the cases was there a decrease of the serum folic acid concentration which could have been induced by the treatment. The serum folic acid concentration was generally low in these old, long-stay hospital patients.

DISCUSSION

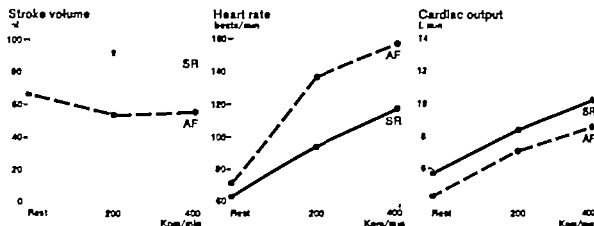
The results of the study indicate that treatment with sulfamethoxazole and trimethoprim is very effective in urinary tract infections and even in those caused by sulfonamide-resistant bacterial strains. The effect is also very good in the treatment of infections caused by Proteus.

A combination of sulfonamide and trimethoprim is obviously indicated in treatment of urinary tract infections caused by sulfonamide-resistant bacterial strains, and the effect of the combined therapy is generally better than that of treatment with trimethoprim alone. The clinical experience in combined therapy with the results from the MIC estimations favours the opinion that sulfonamide and trimethoprim have a synergistic effect. This observation, and the fact that the effect of the combined therapy is bactericidal, makes the treatment very useful as a weapon against urinary tract infections.

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For patients with **atrial fibrillation** regularization to **sinus rhythm** results in a **hemodynamic improvement**



Stroke volume heart rate and cardiac output at rest and during exercise. Mean values of the same 7 men during atrial fibrillation and sinus rhythm (3 males with RHD and 4 without RHD).
Cramér G. Acta Med Scand suppl. 490, 1968.

Cardiac output is increased, enabling the patient to lead a more active life

ithout maintenance treatment the risk of recurrence of fibrillation is great.

Of the available anti-arrhythmic agents, **quinidine** is the best documented drug in maintenance therapy after cardioversion of atrial fibrillation

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COMBINED CHLORAMBUCIL AND PREDNISOLONE TREATMENT OF FIVE PATIENTS WITH WEGENER'S GRANULOMATOSIS

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Abstract. Five patients with Wegener's granulomatosis have been treated with chlorambucil and prednisolone. Three patients are in good general condition 48, 14 and 8 months after commencement of this therapy and with improved or stable renal function. Two patients died. For one of the latter therapy was started late in the course of the disease and could not prevent the fatal outcome. In the other fatal case chlorambucil could not be maintained at an adequate dosage because of complicating hemorrhagic diathesis. On the basis of the experience gained from our own and earlier reported results, a rational treatment of the disease is proposed.

The clinical features as well as pathology and diagnostic criteria of Wegener's granulomatosis have been well described (2, 6, 7, 10, 15). The disease was earlier reported to have an invariably fatal outcome. Thus it was also called "Fatal granulomatosis" (2). This grave view of the disease does not seem to be warranted any longer because of the favourable results obtained through the addition of cytostatic drugs to the therapeutic arsenal (3, 4, 5, 8, 9, 11, 12, 13, 14).

The purpose of the present clinical study is to report on a combined treatment with chlorambucil and prednisolone in five patients with Wegener's granulomatosis. The results of the treatment support an optimistic outlook on the prognosis of this disease.

CASE REPORTS

Case 1

The patient is a previously healthy male storeman, born 1914. In Feb. 1966 he developed symptoms similar to those of an upper respiratory tract infection. During the following two months a progressive loss of hearing and hoarseness developed. Both symptoms have persisted since then. Signs of bilateral otitis media were found.

A chest roentgenogram in April 1966 revealed a 4×4 cm, round, well demarcated parenchymal infiltration in the upper hilar region of the left lung. BP was 170/100, Hb 12.6 g/100 ml. The urinary sediment was normal. ESR, serum creatinine, urinary protein as well as medications since then appear in Fig. 1. Bronchoscopy and thoracoscopy with biopsies showed only unspecific inflammation.

However, a lung neoplasm was suspected and as initial treatment 2 g of cyclophosphamide was given in May. A striking improvement in the patient's condition followed, with almost complete disappearance of the pulmonary infiltrations in August 1966 and normalization of the laboratory findings.

During the autumn of 1966 the patient was able to work for three months. In Dec. 1966 he became tired again, had nosebleedings and headache in the right orbital region. On readmission to hospital in Feb. 1967 he had a right-sided ptosis and miosis and in the right eyeground several hemorrhages and also exudates. Microscopic hematuria and proteinuria were noted.

Due to rapid deterioration of the renal function he was transferred to Medical Department V at Sahlgren's Hospital. He was then pale and tired but had no evident edema and no dyspnea. BP 180/100, Hb 5.9 g/100 ml. Microscopic hematuria was noted. A percutaneous renal biopsy was performed. Marked glomerular changes with thickened membranes and proliferating cells were found. Foci with fibrinoid necrosis and hyaline sclerosis were abundant. The interstitial tissue contained foci with mononuclear cells. Intimal thickening of the vessels was marked, with luminar obstruction. The conclusion was that the findings were consistent with Wegener's granulomatosis.

Chlorambucil and prednisolone treatment was started immediately after admission (Fig. 1) and the patient also received 4×400 ml blood. Urine volumes during the following five days varied between 500 and 1 000 ml/day. Thereafter urine volumes amounted to about 2 l/day and the body weight decreased from 75 to 67 kg. There was also a continuous fall in serum creatinine during the following three months and a striking improvement in his general well-being, as also mirrored in the laboratory findings. Hb, urinary sediment and serum proteins be-

Therapy with prednisolone was started as Wegener's granulomatosis was suspected (Fig. 2).

Because of severe headache and vomiting neurological investigations including cerebral angiography were performed in April 1969 without any salient findings. Later on, protrusion of the optic discs due to increased intracranial pressure was found. Cerebral tumor was excluded after repeated neurological investigations. In Aug. 1969 the patient had to be operated on because of a perforating duodenal ulcer.

In July 1970 the patient was admitted to Medical Department V of Sahlgren Hospital for a therapeutic trial with chlorambucil. Physical examination was noncontributory except for protrusion of the optic discs. Serum creatinine was 1.3 mg/100 ml. Inulin clearance was 58 ml/min. Roentgenograms of the chest showed some regression of the pulmonary infiltrations, while the paranasal sinuses were found to be normal. A new renal biopsy showed focal glomerular scars intermingled with almost normal glomerular tufts. Interstitially chronic, nonspecific inflammation with focal parenchymal atrophy was noted. Chlorambucil was added to the previous treatment with prednisolone (Fig. 2).

Since then the patient has been controlled regularly. Hb, white cell count and platelet count have been normal during treatment, which now has been continued for eight months. After two months of treatment the headache became less severe and after eight months it disappeared. The proteinuria has diminished. Chest roentgenograms in Oct. 1970 were normal. Examination of the eye-grounds in Nov. 1970 showed regression of the optic disc protrusion. Eight months after the start of treatment the patient was in good condition.

Case 4

The patient was a housewife, born 1920, whose elder brother suffered from rheumatoid arthritis. During pregnancy in 1957 a positive Wassermann reaction was noted. The Treponema pallidum immobilization test was negative. In 1960 she had a short attack of disseminated joint pains. In 1965 hoarseness and symptoms of bilateral otitis were noted. Repeated biopsies from the laryngeal mucosa showed a nodular inflammatory reaction. Investigations in her local hospital in July 1966 revealed a positive Wassermann reaction and a slight proteinuria. Serum creatinine and ESR since that time appear in Fig.

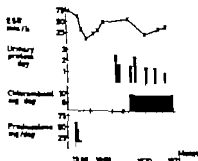


Fig. 2. Case 3. Laboratory findings and medication.

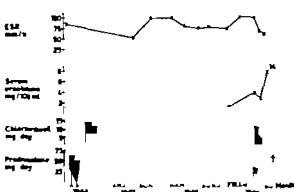


Fig. 3. Case 4. Laboratory findings and medication.

3. Chest roentgenograms were normal. On the suspicion of a systemic immunologic disease prednisolone was instituted (Fig. 3). After initially good results with improvement of the patient's general condition the prednisolone dosage was lowered. During the following months she developed shortness of breath and chest roentgenograms now showed round, well demarcated infiltrations bilaterally.

At readmission in Nov. 1966 Hb, white cell count and platelet count were normal. Microscopic hematuria was noted. An i.v. pyelogram was normal. A biopsy from the nasal mucosa now showed histologic changes typical of Wegener's granulomatosis. Combined therapy with chlorambucil and prednisolone was started, followed by regression of the pulmonary infiltrations. She was then controlled at the Out-patient Department and was in good condition during the spring of 1967. In July 1967 the chlorambucil therapy was withdrawn for an unknown reason, but the prednisolone administration was continued.

In Nov. 1968 a moderate hypertension developed. At readmission the Hb level was then 8.9 g/100 ml and endogeneous creatinine clearance was 75 ml/min. The patient had menorrhagia and the coagulation time was prolonged to 11.5 min. During the spring of 1969 a rise in serum creatinine to 4.4 mg/100 ml was noted. Microscopic hematuria persisted.

On July 10, 1969 her situation was seriously complicated by a spinal subarachnoidal hemorrhage with paraparesis and bladder paresis. After neurosurgical care during two weeks she was admitted to Medical Department V at Sahlgren Hospital and chlorambucil was reinstituted. Due to developing thrombocytopenia and a general bleeding tendency the dosage of this drug could not be maintained at an adequate level and, after temporary improvement of the renal function, uremia progressed and the patient died in her local hospital. No postmortem examination was performed.

Case 5

The patient was a male university student, born 1945, who in April 1966 developed a mucous rhinitis. In Aug. 1966 he was admitted to his local hospital with signs of acute urethritis, conjunctivitis and arthritis of the left knee. The patient's general condition deteriorated, with

signs of renal insufficiency and he was transferred to Medical Department V at Sahlgren Hospital.

On admission the patient was febrile but in good general condition. Granulomatous changes in the nasal cavities were noted. Laboratory findings at admission: Hb 8.8 g/100 ml, ESR 79 mm/h, serum creatinine 7 mg/100 ml. Moderate proteinuria, which excretion between 1 and [illegible] g/d [illegible] and microscopic hematuria were noted. Repeated biopsies from the nasal mucosa showed unspecific chronic proliferative granulomatous changes. Percutaneous renal biopsy failed to give informative material. Repeated roentgenograms of the paranasal sinuses showed increased density of both maxillary sinuses.

The diagnosis of Wegener's granulomatosis was strongly suspected although pulmonary findings were normal and the histological examination of the biopsy material was inconclusive. Therapy with chlorambucil, 15 mg daily, and prednisolone 45 mg daily was started.

On this therapy the granulomatous proliferations in the nasal cavity diminished and the patient's general condition improved. Serum creatinine decreased from 7 to 4 mg/100 ml. ESR, after [illegible] weeks treatment, was 30 mm/h. Roentgenograms of the paranasal sinuses showed marked regression of the pathological findings. After six weeks on therapy the patient went back to his local hospital, now on a daily dosage of [illegible] mg chlorambucil and 3[illegible] mg prednisolone. Three weeks later there was exacerbation of his disease with high, undulating temperature and [illegible] heavy mucous discharge from the nose [illegible] development of severe uremia. Severe anemia and thrombocytopenia developed and the patient died. No post mortem examination was performed.

DISCUSSION

Wegener's granulomatosis is an uncommon disease but may be not as rare as has been generally assumed. Thus during one year (April 1969 – March 1970) reports on about 40 cases were cited in the Index Medicus. During 1966–1970 seven cases have been admitted to our medical department from the western region of Sweden comprising about 1 500 000 inhabitants. Two cases are not accounted for in this study: one patient with advanced uremia at admission did not receive therapy with chlorambucil and prednisolone, the other had symptoms confined to the nasal cavity (midline granuloma) with excellent therapeutic response to chlorambucil and prednisolone.

It is to be suspected that some cases with Wegener's granulomatosis never reach diagnosis. The reason may be a lack of awareness of the disease and the tendency of this disease to engage different organs at separate periods, mimicking at each stage other more common diseases such as upper respiratory tract infections, pulmonary neoplasm and finally glomerulonephritis. Further, even when the suspicion of the correct diagnosis has been awakened, microscopic examinations of biopsy material may be inconclusive. Reports in the literature as well as our own experience show that it is not always possible to establish the diagnosis on the basis of biopsy material (11, 1[illegible]). With lacking histological support it is almost impossible to make a safe diagnosis of Wegener's granulomatosis as long as the disease is confined to the upper respiratory tract. The situation changes, however, when signs of pulmonary and/or renal involvement also appear. The lack of histological evidence must not postpone a provisional diagnosis and the start of vigorous treatment. This is especially important with regard to renal involvement, as the glomerulonephritis may rapidly progress to a stage when "restitutio ad integrum" will be far from possible. Thus, even if successful treatment is possible at advanced stages, as in our case 1 (and in cases reported by Tenberg and Lepper (14) as well as by Hollander and Manning (8)), these patients usually will have reduced kidney function and, as our case 1, develop hypertension. Earlier reported therapeutic trials with corticosteroids alone have been somewhat contradictory (1, 6). Hollander and Manning (8) in their review on [illegible]6 patients with Wegener's granulomatosis treated with corticosteroids, concluded: "Used in high doses early in the course of the disease, corticosteroids prolong the survival time of patients with Wegener's granulomatosis. However, in the late stages of this disorder, when severe renal or pulmonary involvement is already present, a point is reached where even very high dosage of corticosteroids fails to produce remission."

As judged from earlier published results (3, 4, 5, 8, 9, 11, 12, 13, 14) and our own experience it seems quite evident that all cases with Wegener's granulomatosis, including those confined to the upper respiratory tract (also called midline granuloma), should receive treatment with a cytostatic drug. Of these drugs chlorambucil (8, 11) and azathioprine (3, 5, 9, 12, 13) seem to be similarly effective and simple to administer. However, when severe thrombocytopenia occurs with chlorambucil (as in our cases 4 and 5), azathioprine is probably the drug of choice.

Recently a report on methotrexate therapy of Wegener's granulomatosis has been published (4[illegible]

The drug was given once a week without hematologic toxicity In the two reported cases remission of the disease occurred for more than 30 and 26 months, respectively A warning was, however given if methotrexate was to be used in case with reduced renal function, since most of the drug is excreted unchanged by the kidneys. We agreee with the statements (4) that remissions in Wegener's granulomatosis can be achieved with a variety of drugs and that should relapse or failure of response occur with one drug it is comforting to know that another might be effective in this otherwise inexorably fatal disease

Prednisolone should not be given as a single drug, especially not when signs of renal involvement are obvious (8 9) but should be continued when severe side-effects exclude cytostatic drugs temporarily The question whether prednisolone adds significant immunosuppressive effect to that of the cytostatic drugs is as yet not answered. However there are several similarities between the rejection of a transplanted organ and the clinical as well as pathological features of Wegener's granulomatoses. In the transplantation situation the treatment of the rejection benefits from the combined use of cytostatic drugs and prednisolone. Thus, with lacking experience of immunosuppression in the treatment of Wegener's granulomatosis, the above mentioned analogy suggests a combination of a cytostatic drug and prednisolone. Referring to the same analogy it would be wise to maintain the highest possible dose of the cytostatic drug indefinitely This is supported, with one exception (13) by our xperience as well as that of others.

Thus we would recommend as initial dosage of azathioprine 3 mg/kg, or if chlorambucil is preferred 0.2 mg/kg, and 1 mg/kg of prednisolone. The latter is decreased to a maintenance dose of 5–10 mg daily according to the clinical response. The highest possible maintenance dose of azathioprine has to be determined from white cell counts. With chlorambucil, platelet counts are generally more determinative of the maintenance dose than white cell counts.

In our experience ESR is a good indicator of the activity of the disease (cf case 2) It is our belief that, with early diagnosis and combined immunosuppressive treatment with chlorambucil (or azathioprine) and prednisolone followed by careful hematologic controls, the prognosis of Wegener's granulomatosis may be comparatively favorable.

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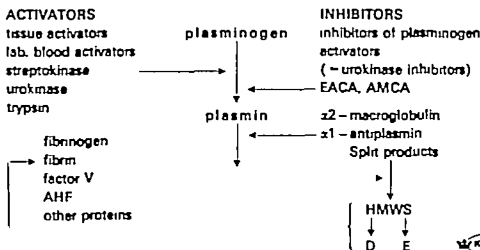
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found wide spread use in a number of haemorrhagic conditions particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sustains various types of haemorrhage in the urinary tract. Cyklokapron produces a haemostatic effect by counteracting the activity of urokinase.

The Swedish investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investigation. Bleeding ceased completely in all the patients in the latter group, as was the case with most of the other patients.

the fibrinolytic system



ANDERSSON, L & NILSSON, I M: AMCA (aminomethyl cyclohexane carboxylic acid, Cyklokapron®) a potent haemostatic agent in urinary tract bleeding. Scand J Urol Nephrol 3 (1969) p 189

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DEATHS FROM RENAL FAILURE IN ABUSERS OF PHENACETIN-CONTAINING DRUGS

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Abstract. Since Feb. 1961 all phenacetin- and paracetamol-containing drugs have in Sweden been subject to prescription and thus less easily obtained. In 1961 a report has been published on the abusers of phenacetin-containing drugs who had died of uraemia at the Medical Department of the Central County Hospital in Jönköping between the years 1954–1960. As a direct continuation the present study has been made of 208 patients, 146 men and 62 women, who died of uraemia in 1960–1970. Among them, 64 men and 22 women were very certainly heavy abusers of phenacetin-containing analgesics. Although phenacetin had been subject to prescription, the deaths of abusers did not decrease but were even doubled in 1966 (15 patients). In 1968 there was a clear regress and in 1970 there were only 3 deaths. A comparison between the two studies shows no changes in either clinical symptoms or patho-anatomical alterations of the kidneys. The male predominance (unlike all other investigations) is due to the severe abuse among the male workers at a factory in Huskvarna. On microscopic examination it is verified that the most common findings are contraction with chronic interstitial nephritis, tubular damage, especially in the lower nephrons, and papillary necrosis or necrobiosis. The present study confirms the risk of renal damage and ensuing fatal uraemia from daily protracted abuse of phenacetin-containing analgesics. It also appears that, although the drug consumption was interrupted, fatal uraemia could develop seven to eight years later. In this study no support has been obtained for the view that bacterial infection is required for the development of renal damage in abusers of analgesics. The fact that Huskvarna workers are now abusing the powder containing 0.50 g phenazone and 0.10 g caffeine, and that during the last few years the number of deaths from uraemia among them has decreased, supports the opinion that this powder does not lead to kidney damage.

In 1961 a study was made (25) of 27 men and 3 women, all heavy abusers of phenacetin-containing analgesics (as a rule a powder containing 0.50 g phenacetin, 0.50 g phenazone, 0.10 g caffeine) who in 1954–1960, died of uraemia at the Central County Hospital in Jönköping—or in three cases elsewhere but after previous treatment at the hospital. The male predominance is unique for this material, all others showing a predominance of women. This peculiarity can be explained by the fact that, in a factory in Huskvarna, very near Jönköping and employing mostly male labour, there was a widespread daily abuse of a certain phenacetin-containing powder in the belief that it would improve working capacity. A thorough investigation was published in 1963 by the factory doctor (13).

In 1961 the National Board of Health (now called the National Board of Health and Social Welfare) decreed that all preparations containing phenacetin and paracetamol, previously available without a prescription, should from then on be subject to prescription. After this decree the consumption of these preparations was reduced to one tenth (7).

As some authors (14, 15, 28, 34) still do not regard it as an established fact that phenacetin causes renal damage, it should be of great interest to find out whether after 1961 there has been any lessening in the number of phenacetin abusers who have died of uraemia at the hospital in Jönköping. Although many abusers may have hoarded the powder it is reasonable to suppose that the abuse had ceased by the end of 1961.

MATERIAL

An examination was made of the case records of all 208 patients who, in 1960–1970, died of uraemia at the Internal Department of the Central County Hospital in Jönköping. As an autopsy was refused in only 4 cases and examination by microscope was made in all but 24, the diagnosis is definite in 180 cases (Table I). The heading "Other diseases of the kidney" covers 8 cases

Table I. *Deaths from uraemia in 1960–1970 at the Department of Internal Medicine, Central County Hospital, Jönköping*

						Analgesic abusers				
Diagnosis	Men	Women	Total	No autopsy	No microscop. examination	Men	Women	Total	No microscop. examination	Huskvarna workers
Chronic extracapillary glomerulonephritis	25	12	37		1	9	3	12		7
Chronic membranous glomerulonephritis	3	1	4							
Chronic interstitial nephritis	29	7	36		4	29	7	36	4	17
Nephrosclerosis	40	4	44		2	20	4	24		15
Chronic pyelonephritis	17	18	35		1	6	8	14	1	6
Diabetic nephropathia	21	8	29	3	9					
Hydronephrosis	7	1	8		3					
Other nephropathies	4	11	15	1	4					
Total	146	62	208	4	4	64	22	86	5	45

genital cystic kidney, 3 of amyloid kidneys, 1 of lower nephron nephrosis, and 1 case of thrombosis of the renal vein. Men predominate, numbering 146 against 62 women.

Table I includes those patients, 64 men and 22 women, who either on their own admission or through information from relatives, were discovered to have abused analgesics, usually containing phenacetin, daily during several years. Among the men, 45 were employed at the factory in Huskvarna. Patients suffering from diabetes or with renal disease possibly caused by some obstructive process (malformation, prostatic hyperplasia) were not included. In the group of abusers of analgesics, autopsies were carried out in all cases and, except for 5 cases of considerably contracted kidneys, all were microscopically examined.

Clinical account

The present investigation is a direct continuation of an earlier study on patients who died from uraemia in 1954–1959 (25). That study contained detailed information about the 30 who abused analgesics. Although preparations containing phenacetin had been subject to prescription since Feb. 1961, this fresh and bigger material collected in 1960–1970, does not differ from the previous one as regards changes in urine and BP. Thus the urine of the patients usually contained only small amounts of protein and a moderate amount of both red and white blood cells. Casts were seldom found, though they appeared abundantly in the kidney at the autopsies. The BP varied considerably. In most patients BP was normal for their age, but there were several cases of hypertension.

Culture of the urine was made more often in the present investigation, in 74 cases out of 86. The result was negative in 36 and positive in 38 cases. The large proportion of positive results is probably due to the inclusion, on this occasion, of 8 women and 6 men with pyelonephritis. This diagnosis was patho-anatomically verified by Ringertz. It was observed that, except for the patients with pyelonephritis, in cases with positive results, microscopic examination showed no signs of either pyelitis or purulent process in the renal parenchyma. As only quantitative determinations were made in the first years of the investigation (27 cases), the positive result may in several cases be due to non-significant bacteriuria. With the exception of the patients with pyelonephritis, this study too thus seems to show that bacterial infection has no main role in the development of contracted kidneys.

The age distribution is also comparatively similar in both studies. This material contains patients who died of contracted kidneys, from 18–40 years through all intermediate ages up to occasional cases of 76–80 years, who thus reached more than the average length of life.

Unlike the previous study, this one does not show such a great male predominance: 64 men against 22 women, only three times as many men compared to nine times as many in 1961. A more persistent investigation on this occasion also of the women's use of analgesics may have contributed to these figures. If the 45 workers in Huskvarna are subtracted, the remaining figures are 19 men and 22 women, which is somewhat more consistent with all other studies, thus showing the predominance of those workers in the present material.

Patho-anatomical findings

In the previous study (25) Ringertz pointed out that it is usual for pathologists to diagnose the kidney condition in cases of phenacetin abuse as pyelonephritic contracted kidney. If by the term pyelonephritis is meant inflammation due to infection ascending from the pelvis, this is a false diagnosis in most cases.

Like other authors (3, 35) Ringertz, too, considers that the common features are above all, the picture of lower nephron damage with abundant granular casts, chronic interstitial inflammation and necrobiosis or necrosis of the medullary papillae. There is also, according to Ringertz, frequently nephrosis in the proximal tubules, which has not been stressed by previous authors.

Also in this study most of the microscopic examina-

Table II. *Deaths from uraemia among analgesic abusers in 1957–1970 at the Department of Internal Medicine Central County Hospital Jönköping*

	Men	Women	Total	Age (y.) Average	Age (y.) Range	Huskvarna orkers	Examined but not deceased at the hospital No autopsy	
1957	4		4	58.5	50–63	4		
1958	7	1	8	53	51–67	6		
1959	7		7	52	43–67	4		
1960	4	1	5	56	39–69	4	1 ♂	
1961	8	3	11	53	40–65	6	2 ♂	1 ♀
1962	7	5	12	53	38–71	6		
1963	5	4	9	49	38–59	4		
1964	6	3	9	52	40–63	5	2 ♂	
1965	5	1	6	56	49–68	3		
1966	13		13	59	43–83	9	1 ♂	1 ♀
1967	5	1	6	62	51–76	4	2 ♂	
1968	4	2	6	52	42–62	1	2 ♂	
1969	4	1	6	59.5	43–83	2		
1970	2	1	3	65	63–70	1		

tions of the kidney preparations were performed or checked by Ringertz (78 cases out of 81). According to his reports the material was then divided into four main groups (Table I). In the largest one, that of chronic interstitial nephritis (29 men and 7 women), the typical changes appear very clearly and other changes, such as arterio-arteriolosclerosis, take minor part. Average age 52.5 years (range 38–69 years).

In the second group (20 men and 4 women) the typical changes are overshadowed by pronounced arterio-arteriolosclerosis (nephrosclerosis). Average age 58 years (range 40–73 years). In the third group (6 men and 8 women) the changes are overshadowed by acute or chronic pyelonephritis. Average age 53 years (range 38–76 years). The fourth group (9 men and 3 women) is composed of patients with the type of changes in the kidneys usually seen in glomerulonephritis. Average age 56.5 years (range 43–83 years). In the whole remaining part of the kidney material (122 cases), which to large extent was microscopically examined by Ringertz or his assistants, there were, except in diabetics, no "typical" changes to be found.

ABUSE OF ANALGESICS AND KIDNEY DAMAGE

As was mentioned above, in Feb. 1961 the Swedish National Board of Health and Social Welfare decreed that all preparations containing phenacetin or paracetamol should be subject to prescription. According to Hood and Bengtsson (16) the consumption of phenacetin-containing tablets and powders went down from 31.4 millions in 1959 to 1.8 million in 1962.

I spite of the lowered consumption of phenacetin-containing drugs, the present material does not show any regress in the number of deaths among abusers of analgesics, but even an increase up to 1966. Only from 1967 onwards is there a noticeable improvement (Table II).

Nor could an immediate decrease in the death rate really be expected. For one thing, a number of patients had probably been hoarding powder and also according to previous experience already established damage of the kidneys will progress although the drug abuse has ceased. Grimlund (13) found that, at creatinine alues of more than 2.5 mg/100 ml, the prognosis is bad and death of uraemia may follow within a few years.

As the workers in Huskvarna were thoroughly informed and followed by the factory doctor it is probable that, after the consumption of possible hoards of the powder all workers stopped taking phenacetin-containing drugs during 1961. All the men in the column of deaths outside hospital (Table II)—except one in 1960—are workers from Huskvarna who after long period at the hospital were discharged and died of severe uraemia at home or in chronic nursing homes. Although in those cases no autopsies were per formed, it is probable that all of them had kidney damage due to abuse of analgesics. If these patients are added to those who died in hospital, the figures for the workers in Huskvarna will be: ten in 1966, six in 1967 three in 1968, two in

1969 and one in 1970. These figures seem to imply that six years after the interruption of the abuse of analgesics a maximal death rate was attained and, after eight years, a definite regress. It may be hoped that from 1970 onwards only occasional deaths will occur

The figures also show that after the interruption of the abuse many patients live for six to seven years, but after that time only a few survive In the present material the average age of the patients who died with contracted kidneys following abuse of analgesics is 55 years, but there are some instances of ages as high as 73–83 years in spite of the abuse Cases are also known in which, in spite of severe abuse no signs of renal damage could be found.

After 1961 a fresh problem has, however developed at the factory in Huskvarna. Many of the workers had become so accustomed to eating powders that, in spite of warnings, they changed to an almost equivalent consumption of a prescription-free powder with 0.50 g phenazone and 0.10 g caffeine A voluntary experiment is in progress, testing the possible damaging effect on the kidneys of phenazone-caffeine powder

DISCUSSION

authors do not regard it as sufficiently lished that protracted phenacetin abuse is the cause of the increasing occurrence of chronic interstitial nephritis with papillary necroses (14 15 28 34)

Since the first publication by Spühler and Zollinger (32) there has, however appeared a vast number of reports, especially from Switzerland, Denmark and Sweden (see references in 6, 13) on the connection between the increased appearance of diffuse interstitial nephritis with papillary necroses and the growing abuse of analgesics. In recent years similar reports have appeared from Britain (5 9 22, 26, 29) Germany (24 30) Australia (17 19 23) Canada (11 20 21 27) and USA (10 31) As phenacetin was included in nearly all the compound preparations that were abused, whereas other ingredients varied considerably phenacetin as the common factor was suspected as, or considered to be the most important cause of the kidney damage.

Why phenacetin is injurious to the kidneys on a daily consumption of many years duration is, at present, not known. In 1966 Haley (14) made a critical survey of the investigations which had been carried out regarding the administration to humans and animals of large quantities of phenacetin. The animal experiments, which were often extended into months and years, comprised certain series where bacteria were introduced simultaneously He concluded that, at present, it could not be established that phenacetin causes damage to the kidneys. Haley attaches great importance to the fact that the abused analgesics are all compound preparations and he calls for an extensive investigation of all components before accepting the nephrotoxic qualities of phenacetin.

In 1969 Bengtsson (7) defended her opinion of phenacetin as the pharmaceutical cause of the renal damage She points out that salicylic acid, though experimentally shown to irritate the kidneys and in spite of its intense use in rheumatoid arthritis, has occasioned no large outbreaks of interstitial nephritis. In Sweden the analgesics causing fatal uraemia very frequently contain only phenazone and caffeine beside phenacetin. In her own patient Bengtsson found no injury to the kidneys after protracted consumption of phenazone and caffeine alone and she has, so far found only one case in the literature (4).

In 196_ Bengtsson (6) wrote: At present it may be concluded from clinical and experimental data that prolonged consumption of phenacetin makes the kidney suspectible to infection. It can also be stated regardless of which came first, the bacteria or the phenacetin once the kidney is diseased excessive phenacetin consumption frequently causes renal papillary necrosis.

Unlike all other similar reports, the present one on abusers of analgesics who died of uraemia at th Central County Hospital of Jönköping shows a predominance of men—27 men and 3 women in the first investigation and 64 men and 22 women in the second. The reason for this is the large incidence of renal damage among the 3 000 mostly male workers at a factory in Huskvarna. From 1954 to 1970 60 Huskvarna workers died at the hospital of chronic interstitial nephritis and papillary necroses. The existence of a severe abuse of phenacetin-containing powders until 1961 at this factory is well established. Thus it cannot be doubted that daily consumption for many years of drugs containing 0.50 g phenacetin, 0.50 g phenazone, and 0.10 g caf

feine may result in kidney damage severe enough to lead to death from uraemia.

Whether the phenacetin by itself is nephrotoxic, or only when combined with phenazone or caffeine does not, however appear from the present investigation. Haley's critical opinions should thus receive due consideration as long as animal experiments have produced no kidney damage from phenacetin by itself

According to Grimlund there is now in spite of warnings, an extensive abuse of powders containing 0.50 g phenazone and 0 10 g caffeine among the male workers in Huskvarna. Therefore it will probably not be many years before it becomes clear whether this powder too is nephrotoxic.

As seen in Table II, a clear decrease of deaths among Huskvarna workers has been noticed in the last few years. This fact may—even now—indicate that the abuse of powder containing phenazone and caffeine does not result in kidney damage For definite information it will, however be necessary to await examinations of the kidneys of patients who have not previously abused phenacetin-containing drugs.

Bengtsson (6) points out that a bacterial infection is required in addition to the phenacetin abuse for the development of severe renal damage. Her material shows a predominance of women with clinical symptoms of acute or chronic pyelonephritis. This second report on 86 abusers of analgesics contains 22 women, 8 of whom also have a patho-anatomically verified pyelonephritis. In this group the bacterial infection was, of course, of great importance For the 36 cases with no history of infection of the urinary tract, no signs of pyelonephritis as defined on microscopic examination by Ringertz, and negative result in culture of urine, bacterial infection will not have had the same decisive importance. It may even be questioned whether there was any bacterial involvement at all in these cases.

In spite of the negative reports from animal experiments, added evidence of the damaging effects of phenacetin on the kidneys has lately appeared with the discovery that phenacetin abusers develop renal cancer more frequently than a normal population (1 2, 8) It is suspected that the phenacetin metabolite, 2-hydroxyphenetidine, has a carcinogenous effect, as it is chemically related to known carcinogenous products.

In this material there are three families, and in that published by Grimlund (13) nine, in which two to four members died of uraemia following abuse of analgesics. As we know that it is possible to abuse analgesics for long periods without damage to the kidneys, this high frequency of renal damage in one family may possibly indicate the existence also of a hereditary element, not merely of an epidemic bad habit within the family

The importance of a hereditary element may possibly also be indicated by the not infrequent cases of migraine among abusers of analgesics with kidney damage In this material 4 women and 1 man complained of severe migraine. This is, of course, a hereditary disease and the tendency to angiospasm might conceivably contribute to the development of kidney damage, as pointed out by Ask-Upmark (3) It should, however also be kept in mind that headache by itself could induce abuse of analgesics.

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Fig. 1 (a, b) Emission patterns over feet and hands in control subject with no signs of peripheral circulatory disturbances.

h are amplified and modified to give picture on an illoscope screen. This visible picture is then photographed. An isotherm set at various temperature level is introduced into the infrared picture on the screen in order to obtain an objective measure of differences in emission levels.

The measurements were performed in a room especially devised for thermography. The room is air-conditioned and the temperature maintained constant at 18.5 0 °C. Direct sunlight and all possible draughts are excluded. Before the thermographic examination the patient rested in the examining room for 15 min to obtain thermal equilibrium.

Clinical method

All patients were routinely examined for the following diabetic manifestations.

1. Retinopathy was graded as follows: 0 no changes, + = microaneurysms, microaneurysms and haemorrhages with or without exudate + proliferations retinopathy.

2. Nephropathy. Constant proteinuria without signs of infection (negative bacterial urine culture) was attributed to diabetic nephropathy.

3. Peripheral angiopathy.) The skin temperature was measured on the toes after indirect heating according to Bratteland et al. (7). b) Oscillometry was performed on the lower limbs using von Reck-inghausen oscillometer. c) Arterial calcifications in the legs were looked for in plain radiograms. d) Skin biopsy specimens were taken from the dorsum of the foot and the wall thickness of capillaries and venules was estimated according to Sjö Söderbergh et al. (10). T o abnormal examinations are required for diagnosis f peripheral angiopathy.

4. Neuropathy. Besides routine neurological examination, lectromyography (EMG) as carried out and the conduction velocity in a peripheral motor nerv of the lower extremity was determined as described previously ( ). Diabetic neuropathy as diagnosed on at least to typical neurological signs (g. bi teral Achilles areflexia, diminished vibratic sense in the legs, alternatively abnormal EMG and conducting velocity).

The ophthalmoscopic examinations were performed in the Department of Ophthalmology and the skin temperature measurements in the Department of Clinical Physiology.

RESULTS

Control series

The appearance of the thermographic picture was almost constant in the control subjects, with a

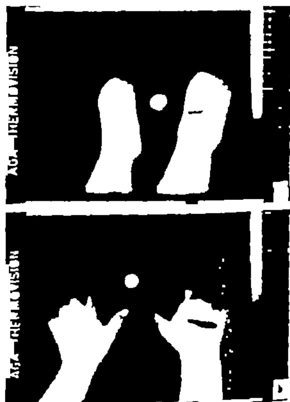


Fig. (a, b) Typical emission pattern over diabetic feet and hands with no signs of local gangrene. The strongly reduced emission over fingers and toes is frequent in diabetic patients.

regular pattern of hot and cold spots. The thermographic picture showed well defined fingers and toes with high emission over the tips (Fig. 1 *a* and *b*). Seven of the 27 healthy subjects showed reduced emission over feet and 5 over the hands. None of the controls exhibited any asymmetry between right and left feet and hands.

Patients with diabetes

All patients with diabetes had some sort of thermographic abnormality or asymmetry.

Reduced emission patterns over the feet were present in 42 of the 47 patients. The abnormal thermographic pattern appeared as an abrupt change from warmth to cold over the toes or metatarsophalangeal regions, sometimes with a tapering-off of higher temperature to very cold toes (Fig. 2). In 30 diabetic patients differences were recorded between right and left feet and a definitely asymmetric pattern was shown on the thermograph, with loss of emission over one or more toes of one foot (Fig. 3 *a*).

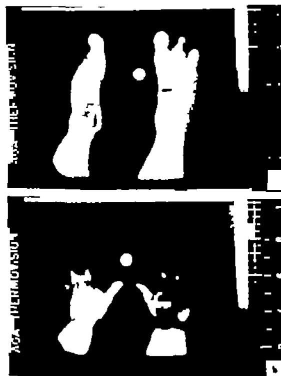


Fig. 3 (*a*, *b*) Pronounced asymmetric emission patterns over diabetics' feet and hands with no signs of local gangrene. Note the irregular pattern with strongly reduced emission over some fingers and toes.

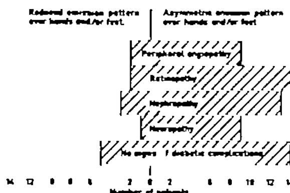


Fig. 4 The thermographic picture in relation to the incidence of diabetic complications.

The reduced emission patterns over the hands were less pronounced and less extensiv than those over the feet. Reduced emission patterns were recorded in 27 and asymmetric patterns in 15 diabetic patients (Figs. b and 3 *b*).

The mean duration of the disease in the group of 35 patients with asymmetric thermographic pattern over the hands and/or feet was 14.9 ± 8.7 years compared with the group of 12 patients with diabetes exhibiting reduced thermographic pat tern, in which the average duration was 9 7 ± 5.2 years. When the patient series was divided into subgroups and the thermographic picture related to the vascular and neurological complications, there was no striking correlation (Fig. 4).

DISCUSSION

The skin as medium of heat regulation makes static temperatures difficult to evaluate. Infrared emission patterns over hands and feet demonstrate asymmetries, which were absent in the control subjects. Over both hands and feet the thermographic findings of the diabetic patients were in most cases distinctive, with an asymmetric decrease in the emission levels over one or more toes or fingers. The thermograms resembled those of patients with Raynaud's disease with gangrene in finger tips and toes (Fig. 5).

In recent years morphological changes of arterioles, capillaries and venules have been demonstrated in skin and muscles in patients with diabetes, as well as in the kidney and retina. The changes are patchy. Histologically vessels with markedly thickened walls may be observed next



Fig. 5. A 58-year-old man with Raynaud' disease. Multiple ulcerations on toes of both feet and corresponding reduced emission patterns.

to vessels with thin, apparently normal walls. This variability may express functional differences between the various vascular segments.

The number of patients examined in this study is too small to permit statistical analysis of the relationship between the thermographic picture and diabetic complications. Diabetic patients with emission patterns, which also were present in the controls, often had several signs of vascular and neurological involvement. On the other hand, some diabetic patients with definitely pathological emission patterns showed no signs of diabetic complications. This apparent confusion might be explained by our coarse clinical methods and the patchy distribution of diabetic microangiopathy. Since vascular changes are considered to be related to the duration of the disease it is interesting to note that diabetics with asymmetries presented a significantly longer mean duration of diabetes than those without such asymmetries. The abnormal thermograms of patients with diabetes are interpreted as signs of circulatory disturbances in hands and feet. With the present technique it is impossible to judge whether these disturbances reflect a true diabetic microangiopathy or are signs of autonomic neuropathy.

In order to be able to interpret the pathological thermograms over hands and feet in patients with diabetes mellitus, it is necessary to study such patients for a long time. It is also necessary to record the thermograms frequently and to correlate the findings to physiological studies of nerves and vessels. The infrared emission from the skin bears a direct relationship to the surface temperature (8) which under resting conditions is thought to reflect the blood flow in the underlying tissues in hands and feet (11).

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VAGAL FUNCTION IN PATIENTS WITH DIABETIC NEUROPATHY

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Abstract The gastric secretory response to insulin administration has been investigated in eleven diabetic patients in order to study their vagal function. The mean duration of the disease was 16 years. One patient suffered from diarrhoea. The other diabetic patients were free of symptoms from the gastrointestinal tract. From the criteria in the literature for surgical vagotomy some patients with long duration of the disease and with severe signs of vascular and neurological involvement exhibited impaired vagal function. This supports the theory that impaired vagal function is the cause of disturbances of the gastrointestinal tract in diabetes mellitus.

Disturbances of the gastrointestinal tract are common in diabetes mellitus. Anorexia and vomiting often occur in patients with hyperglycaemia. Dilatation of the stomach, gastritis and haematemesis are also seen in diabetic keto-acidosis.

Gastrointestinal complications sometimes affect long-term diabetic patients. Dyspepsia with vomiting and retarded gastric emptying are seen in some patients (3, 10) and diarrhoea in others (16). Common findings in patients with gastrointestinal complications are diabetic angiopathy with retinopathy, nephropathy and neuropathy in peripheral nerves. Angiopathy in the wall of the stomach and intestines has also been found (1, 4).

The pathogenesis of the gastrointestinal tract disturbances is obscure. Diabetic neuropathy of the autonomic nervous system has been proposed on slender evidence (16) and some authors suggest an abnormal bacterial flora in the gut as a contributory factor (6, 17). These patients have often other signs of autonomic disturbances such as postural hypotension, impotence and urinary bladder dysfunction.

In a previous publication, Dotevall (3) suggested that the degenerative changes in the nervous system would bring about the same functional condition as vagotomy. However, very few investigations have been performed on the neuropathological findings in diabetes mellitus. Pathological data are unsatisfactory. Only Kristensson et al. (11) have reported changes in the vagal nerves in some diabetic patients with long duration. Hensley and Soergel (7) have investigated 3 diabetic patients with regard to neuropathological changes. Abnormal findings were seen in prevertebral and paravertebral ganglia but not in the vagal nerves.

The ability of insulin to stimulate gastric secretion depends on its glucopenic effect. Intracerebral glucopenia stimulates the vagus centre, which in turn excites the gastric glands by way of the vagus nerves. The purpose of the present investigation was to study the effect of insulin hypoglycaemia on gastric secretion in patients with diabetes mellitus and so to investigate the vagal function.

MATERIAL AND METHODS

The investigation was performed on 11 diabetics with varying durations of diabetes (range 2-26 years, mean 16 years). All patients were on insulin and diet consisting of 50% carbohydrate, 20% protein and 30% fat. All patients were clinically in satisfactory metabolic state.

Clinical methods

All patients were admitted to the hospital during the investigation and underwent routine medical examination.

Retinopathy was graded as follows: 0 = no changes; 1+ = microaneurysms; 2+ = microaneurysms and bleeding with or without exudate; 3+ = proliferative retinitis.

Neuropathy. Besides routine neurological examination, electromyography was carried out. At least two neuro-

Table I. *Clinical findings*

Case no.	Age (y)	Duration of disease (y)	Neuro-pathy	Retino-pathy
1	21	6	+	0
2	34	23	+	0
3	39	26	+	1+
4	41	23	+	2+
5	45	18	+	2+
6	46	13	+	1+
7	47	12	0	0
8	49	13	0	0
9	50	2	0	0
10	61	20	+	2+
11	61	18	+	2+

logical signs typical of diabetic neuropathy had to be present for the diagnosis of neuropathy

Clinical data on the patients are given in Table I

Procedure and laboratory methods

Gastric secretory studies were performed in the morning after 12 hours' fasting and abstention from smoking. *A nasogastric tube (Salem Sump Tube) was introduced.* The patient was in a semi-supine position and was instructed not to swallow saliva. Continuous drainage of the stomach was carried out by suction with pump giving the pressure of −50 mmHg intermittently. Injec*tion of air and intermittent suction by means of syringe* was used to prevent blocking of the tube. Gastric juice was pooled for individual 15 min periods. The volume gastric juice was measured in ml, the pH recorded, , the concentration of hydrochloric acid, determined by with N/10 NaOH to pH 7.0. The output of 'hydrochlori. acid in mEq/15 min was calculated as a product of the volume (l) and concentration (mEq/l).

Table II *Blood sugar values initially and afte insulin administration*

Case no	Initial (mg/100 ml)	Lowest (mg/100 ml)	Time from insulin administration to blood sugar value below 80 mg/100 ml (min)
1	173	30	30
	84	24	30
3	260	80	60
4	67	30	0
5	103	33	60
6	172	35	60
7	213	47	90
8	94	22	30
9	147	17	30
10	91	39	30
11	266	27	60

In 4 patients the proteolytic activity was determined by Hunt's method (9).

Before the study all patients received their regular dose of insulin. When the gastric secretory studies started, samples of capillary blood were obtained for blood sugar determination by the glucose-oxidase method (12). In order to get immediate but rough information, the b ood sugar was approximated with the aid of test strips (Dextrostix, Ames) which were used simultaneously with the glucose-oxidase method.

After 60 min collection of basal secretion and determination of blood sugar values, 20 IU of insulin was given i.v. I one subject, however only 16 IU was given because of a slightly low blood sugar value. The secretion was investigated further and blood sugar determined every 30 min thereafter. In 3 patients, 8, 10 and 20 IU of insulin, respectively were given 30–60 min after the first i. dose before hypoglycaemia was induced. Clinical signs of hypoglycaemia were seen in all patients.

In 7 patients the ordinary pentagastrin test was per formed on another day (6 μg/kg b.wt. subcutaneously) (Table III).

The ophthalmoscopic examinations ere performed in the Department of Ophthalmology of this hospital.

RESULTS

In this study the following criteria were used for positive response (intact vagal function) using the insulin test.

1) An increase of acidity 20 mEq/l within 2 hours of the insulin injection or 10 mEq/l if basal secretion was anacid (8). 2) Increase in volume during hypoglycaemia compared with pre-insulin periods (18) 3) The same criteria as used by Hollander A positive response within 45 min is designated as an early positive response and one occurring after 45–120 min as a late positive response, which latter has been assumed to indicate a disturbed vagal function (15) 4) Increase in pepsin output during insulin-hypoglycaemia compared with pre-insulin periods (5) 5) The ratio of response to insulin and pentagastrin for acid output (peak half-hour) For intact func tioning vagus the ratio ranges from 45–165 % (19).

Table II shows initial blood sugar values and the lowest blood sugar value recorded. The time in minutes from insulin administration to blood sugar value below 80 mg/100 ml is also noted.

Two of the diabetic patients did not fulfil the criteria for intact vagal function according to the criteria of Hollander (8) Waddell (18) and Ross and Kay (15) In one of these patients the pepsin output was investigated. No significant in-

crease in pepsin output was seen during hypoglycaemia. Three of the other 9 diabetics had an early and 6 a late insulin response. Besides the insulin test the common pentagastrin test was performed in 7 patients. In 2 patients the peak insulin response was 100 and 63% of the pentagastrin response. One patient had a borderline value of 42% and the other 4 diabetics ranged from 25 to 0% of the peak insulin/pentagastrin response (Table III).

DISCUSSION

Eleven patients with diabetes mellitus have been studied by a modified hypoglycaemia test according to Hollander. The purpose of the study was to investigate the vagal nerve function of the stomach. In 2 patients prolonged hypoglycaemia did not increase either acidity, volume or acid output. One of these patients had severe gastrointestinal disturbances. This patient had basal acid secretion of 6.8 mEq/h and after pentagastrin 29 mEq/h. Both values are high for his age and weight (2). The pepsin output in this subject was not increased significantly by hypoglycaemia. The other patient had low secretory response after pentagastrin stimulation (1.1 mEq/h). During insulin hypoglycaemia, however, the pH of the gastric content was 7.4 and 7.2 compared with 7.0 for basal gastric juice. These patients had impaired vagal function interpreted according to all criteria for complete vagotomy.

In nine patients hypoglycaemia induced an increased gastric acid secretion. In 3 of them the increase in acid concentration, volume and output came within 45 min and in 6 between 45 and 120 min. According to the criteria of Ross and Kay the latter diabetics had a late positive response, indicating some degree of damage of the vagal trunk. However, in some patients with high initial blood sugar values the blood sugar level never fell below 50 mg% in the first half-hour after the injection, which makes the results when using these criteria difficult to interpret. In diabetics, who often have low secretory capacity, it seems more appropriate to compare the response to insulin with the response to pentagastrin for acid (19). This has been claimed to be an index of the receptor pathways. This insulin test gives a quantitative estimation of the vagal function. Seven patients were tested. Three diabetics with

Table III. *Acid output in gastric juice basally and after insulin and pentagastrin administration*

Vagal function interpreted according to criteria of Hollander and Waddell (positive response = +, negative response = –) and Venables & Johnston
PAO_I = peak acid output after insulin, PAO_{PG} = peak acid output after pentagastrin

Case no.	Basally (mEq/h)	After insulin (mEq/2 h)	After pentagastrin (6 µg/kg)	Response	PAO_I/PAO_{PG} (%)
1	5.0	28.1	26.4	+	36
2	1.4	12.7	20.5	+	25
3	0	1.1	11.7	+	8
4	0.7	5.2*			
5	1.0	5.7		+	
6	0	0	1.1	–	0
7	1.7	7.2	24.7	–	42
8	0.5	11.3	7.6	–	100
9	5.4	34.6		+	
10	0.1	5.7		+	
11	6.8	2.5	29	–	0

* Followed for 90 min.

long duration of the disease and with severe signs of vascular and neurological involvement had values of PAO_I/PAO_{PG} in the range for patients with surgical vagotomy indicating decreased function of the vagal trunk.

Changes in the autonomous nervous system in diabetes have been suggested as a cause of disturbances in the gastrointestinal tract and have also been found in neuropathological studies (7, 11, 14).

The present study supports the theory that vagal function is impaired in some patients with diabetes mellitus and this may be a cause of retarded gastric emptying. The peristaltic power of the stomach is also reduced in diabetics with severe late complications compared with diabetics without complications. This also indicates a vagal nerve lesion.

Diabetic diarrhoea has been extensively studied by Whalen et al. (20). In their patients with diarrhoea neither gastric atony nor impaired oesophageal motor function was seen. The normal intestinal response to 1-epinephrine, 1-norepinephrine and metacholine indicated intact sympathetic and parasympathetic pathways in the gut. However, lesions in the vagal system on other levels cannot be ruled out by this study. Malins and Mayne (13) found a normal histological pic

ture in biopsy specimens from the jejunal mucosa and stressed the association between diabetic diarrhoea and autonomous neuropathy

The diarrhoea in diabetic patients with neuropathy also has many clinical and laboratory features in common with postvagotomy diarrhoea. Both types are intermittent and characterized by frequent watery stools, often impossible to control voluntarily Steatorrhoea and other types of malabsorption may occur in both types. In diabetic diarrhoea, treatment with anticholinergics cholinergics, corticosteroids, pancreatic ferments, vitamin B_{12} and broad-spectrum antibiotics has been tried without convincing improvement.

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DIGITOXIN STUDIES

Serum Concentration during Digitalization Maintenance Therapy and Withdrawal. Estimation of Proper Maintenance Dose

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Abstract. Serum digitoxin has been determined in 144 patients by modified Rb-ATPase method. Mean digitoxin concentration during maintenance therapy increased with the daily dose: 0.036 mg day – 10.8 ng/ml, 0.05 mg/day – 11.2 ng ml, 0.071 mg/day – 16.1 ng/ml, 0.1 mg day – 18.1 ng/ml. The increase in serum after different loading doses is presented. After withdrawal of maintenance therapy in 20 patients the mean serum half life was 6.9 days (S.D. = 2.7 days). The correlation serum half life—serum creatinine was significant. After determination of serum concentration and serum half life in particular patient is possible to calculate the proper maintenance dose which should keep his total body store of digitoxin at an optimal value.

During the last years new methods have been introduced for determination of serum digitalis concentration (Table III). Here we report our experience with digitoxin determination during digitalization, maintenance therapy and withdrawal.

METHODS

Analyses. The determination is based on the ability of digitalis glycosides to inhibit ion flux across red cell membranes. The original method by Lowenstein and Corrill (15) has been greatly modified to obtain increased accuracy precision and capacity (analytical coefficient of variation 8%, time spent on each sample about 10 min) (7).

Patients and blood samples. Serum samples were obtained from adult patients at the Medical Department, Drammen Hospital. In the steady state studies, samples have usually been drawn on the second or third hospital day and regardless of last dose or meals. The patients have been asked about their previous digitoxin medication on admission and before blood sampling. Only patients giving the same answer on these two occasions and with constant dose for at least 30 days have been included in the steady state groups. The patients had serum creatinine below 2.0 mg 100 ml and no other diseases which might interfere with digitoxin absorption, distribution in the body metabolism or elimination (malabsorption, nephrosis, liver and thyroid dysfunction). The serum albumin was only controlled in patients with suspected liver disease or nephrosis. Serum was separated from erythrocytes within hours, and the samples have been stored at +4°C for few days before analyses. Repeated samples from the same patient were usually kept at −20°C until all samples could be analyzed in the same series.

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RESULTS AND COMMENTS

Maintenance therapy

Table I shows that the serum concentration increases with the doses. The increase is not linear. The observed range is surprisingly great, and an *unusual high or low value may be found with* different doses. There was slight difference in mean serum concentration for men and women, this was explained by small difference in weight.

Correlation serum concentration – creatinine – weight – age

In the group of 81 patients using 0.1 mg digitoxin each day correlations were calculated and are presented in Table I.

Constancy of serum concentration of individual patients during maintenance therapy (Fig. 1 *a*)

Blood samples were obtained at regular intervals from 22 patients with stable congestive heart failure. Mean concentration for the first set of samples was 15.78 ng ml compared with 15.66 ng/ml in the last set of samples.

ture in biopsy specimens from the jejunal mucosa and stressed the association between diabetic diarrhoea and autonomous neuropathy

The diarrhoea in diabetic patients with neuropathy also has many clinical and laboratory features in common with postvagotomy diarrhoea. Both types are intermittent and characterized by frequent watery stools, often impossible to control voluntarily Steatorrhoea and other types of malabsorption may occur in both types. In diabetic diarrhoea, treatment with anticholinergics, cholinergics, corticosteroids, pancreatic ferments, vitamin B_{12} and broad-spectrum antibiotics has been tried without convincing improvement.

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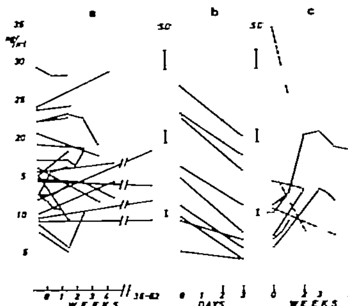


Fig. 1 (*a*) Constancy of serum concentration of 22 patients during maintenance therapy. Analytical S.D. in the middle columns. (*b*) Decrease in serum concentration after withdrawal for two days. 9 patients. (*c*) Serum concentration after increase in dose from 0.05 to 0.1 mg day (——). 4 patients. Decrease in dose from 0.1 to 0.05 mg day (– – –). 4 patients.

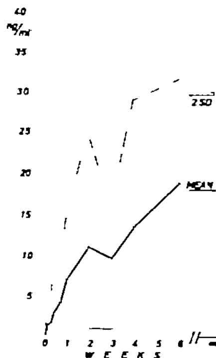


Fig. 2. Increase in serum concentration after digitalization with 0.1 mg day. Mean value of 10 patients (——), with largest and smallest observed values (—), and theoretical increase ( ), are demonstrated. Mean value – S.D. for 0.1 mg maintenance group at right Y-axis.

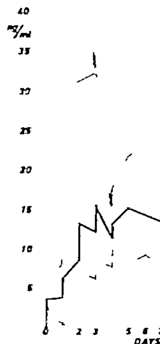


Fig. 3 Digitalization with 0.3 mg for 4 days followed by 0.1 mg day. Blood samples before and one hour after each dose. Mean value and largest smallest values for 19 patients.



Fig 4 Digitalization with 0.6 mg for two days followed by 0.1 mg/day Mean value and largest/smallest values for 16 patients.



Fig 5 Digitalization with 0.9 mg first day 0.3 mg second day next days 0.1 mg. Mean value and largest/smallest values for 9 patients.



Fig 6 Digitalization with 1.. mg first day followed by 0.1 mg day Mean value and largest/smallest values for 10 patients.

significant differences) At this time the mean serum concentration was 13.4 ng/ml, somewhat lower than the observed mean value during 0.1 mg maintenance treatment (18.1 ng/ml)

Serum half life ($T/2$)

Repeated samples were obtained after withdrawal of medication on 23 occasions and also after 4 serious acute intoxications. Mean $T/2$ was 6.8 days. Twenty of these patients had been on constant maintenance therapy with 0.1 mg/day In this group further calculations were made (Table II) The variation in $T/2$ was large from patient to patient, and no specific medication (diuretics or phenobarbital) was found which differentiates the patients with rapid or slow elimination. The observed $T/2$ for 4 young patients with acute intoxication was within the range found for patients with maintenance therapy and saturated digitoxin stores in the body There was no difference in $T/2$ for males and females.

Intoxication

Several patients with clinical suspicion of intoxication were observed. However few of these

Table II. *Correlations T/2–serum concentration–creatinine–age–weight*

For 20 patients on maintenance therapy

	Mean	S D	Observed range Min.	Observed range Max.
T/2 (d)	6.855	2 67	2.4	11.5
Concentration at withdrawal (ng/ml)	19.9	6 30	5 5	31 0
Serum creatinine (mg/100 ml)	1 07	0 25	0 5	1 4
Age (y.)	64 5	18.3	19	87
Weight (kg)	60.7	10.7	45	89

	Regression line	*p*	
T/2–concentration	1.3+0 28 concentration	0 003	0 63
T/2–creatinine	0 87+5.6 creatinine	0 02	0 53
T/2–age	2.4+0 068 years	0 04	0 47
T/2–weight	7.5+0 010 kg	0.86	0 04

patients really had serum values above mean + 2 S D = 30 ng/ml. Conversely other patients presented higher serum values after an overdosage at home, but without the usual symptoms and signs of intoxication. This problem will be discussed in a later paper In our experience only serum concentrations above 35–40 ng/ml together with symptoms and clinical signs (ECG) of intoxication should be regarded as true intoxication

DISCUSSION

Determination of digitoxin has now been successfully tried by various methods. The serum concentration for patients on maintenance therapy is reported to be about 10–50 ng/ml (Table III) At present the most rapid determination is the direct ATPase method measuring phosphate release (1 2) Otherwise the choice between the methods must depend on experience and equipment (7)

The most striking result in Tables I and III is the wide range observed for patients on maintenance therapy Some of the extreme values may be explained by uncontrolled medication at home by old patients using a variety of other tablets. However most of the observed variations seem to be real, and usually the surprisingly low or high values remain stable after hospitalization with constant medication.

The observed serum concentration represents the final result of 4 factors—absorption, distribution within the body metabolism and elimination. The absorption of digitoxin should be nearly 100 % in normal individuals, but is decreased

Table III. *Reported values of serum digitoxin determinations by different methods*

Method (reference)		Dose (mg/day)	No. of pats.	Mean serum concentration (ng/ml)	Observed range (ng/ml)	Precision ( )	Elimination	Toxic concentration (ng/ml)
ATPase erythrocyte bioassay								
Original method	(15)			25	10–50		T/2 = 1 6–4 0 d	
Modification	(19)		21	19	0–36			> 39–51
Modification	(20)		26	33				
Modification	(7)	0.036	6	11			T/2 6 9 d.	
		0 05	33	11		8		40?
		0 071	24	16			S D 2.7 d	
		0.1	81	18				
Direct ATPase bioassay–	(2)		20		12–28			
phosphate release	(1)	0 05	10	13	2 22			
	(1)	0 1	115	22	2 50		1 58 ng/ml, d.	> 41
		0.15	17	30	10–45			
		0.2	4	47	43–50			
Double isotope dilution derivative	(17)		20	20	10–49	4–20	T/2 4 3–6 4 d	> 43–67
Radioimmunoassay	(18)		19		4–60	11 32		
Radioimmunoassay	(21)	0 1			4–30	5		

Table IV *Theoretical variations in serum concentration with different doses Starting concentration in parentheses*

For calculations see text

Change to maintenance dose D_2 (mg)	Maintenance dose D_1 (mg) 0.1	0.071	0.05	0 036
	New steady state serum concentration (ng/ml)			
0.1	(18.1)	22 5	22.3	30.1
0 071	12.9	(16.1)	15 9	21.5
0.05	9 1	11.2	(11.2)	15 1
0 036	6.5	8.0	8 0	(10.8)

when clinical malabsorption is present (6, 9 10 own observations) There is also reason to suspect some variations in absorption as an explanation of the large variations in serum concentration after loading doses and during maintenance therapy

Our knowledge about the distribution in the body is scarce and only a few studies in selected patients have been performed (3) The serum-myocardial ratio has been reported to be about 1 30–40 for digoxin (3 4) The extensive albumin binding of digitoxin may indicate that the ratio for digitoxin differs from that for digoxin 16, 17) This has not yet been investigated. l serum albumin may give an apparent w serum concentration of digitoxin (own observations) Whether this is accompanied by low myocardial concentration and decreased inotropic effect is not known.

Further the sensitivity of the myocardium may change For instance, it is obvious that a somewhat high serum digitoxin concentration of 35 ng/ml may have different significance in the presence of variations in potassium, calcium or magnesium concentration, O_2 and CO_2 tension, acid-base balance or other unknown factors which interfere with cell membranes and intracellular environment (5)

The metabolism of digitoxin is complex and many of the 20 metabolites including digoxin are cardioactive With methods that are digitoxin-specific this may give lower serum values compared with ATPase methods, which probably measure all cardioactive and ATPase inhibiting glycosides. The present reported values do not indicate any great difference between digitoxin-specific methods and other principles of determination. This difference should be more obvious in $T/2$ studies.

The elimination of digitoxin and metabolites in the normal state and by heart patients has also been insufficiently studied. Serum determination is certainly better than analysis of digitoxin and metabolites in urine (11)

In this report most of the differences in mean serum concentration after different doses were significant (Table I) From these differences some important calculations can be made The relationship between serum concentration (C) and maintenance dose (D) can be calculated according to equation 1 (8, 12, 13 22)

$$C = \frac{F \cdot D \cdot T/2}{V \cdot \ln 2 \cdot t} \qquad (1)$$

(F = fraction of dose absorbed, $T/2$ = serum half life V = apparent volume of distribution of the drug, t = interval between the doses) If we change the maintenance dose for a particular patient from D to D' his new serum concentration C' would be.

$$\frac{C'}{C} = \frac{\dfrac{F' \cdot D' \cdot T/2'}{V' \cdot \ln 2 \cdot t'}}{\dfrac{F \cdot D \cdot T/2}{V \cdot \ln 2 \cdot t}} = \frac{D'}{D} \qquad (2)$$

$(F = F' \quad T/2 = T/2' \quad V = V' \quad t = t')$.

The results of varying the doses for the patients with mean serum concentrations according to Table I are presented in Table IV The first column shows that a decrease in dose gives calculated values which are significantly lower than the real mean serum concentrations observed for the 0.071 0.05 and 0.036 mg groups. This indicates that the patients in the last three groups are somewhat different from the 0.1 mg group and the difference may be in $T/2$ or in the fraction of absorption.

In the maintenance dose studies blood samples were drawn regardless of the last dose given. The failure induced by such uncontrolled sampling can be estimated. In the loading dose studies (with empty stomach) maximal serum concentration was obtained after 45–60 min Per 0.1 mg digitoxin the average serum increase was ~11 ng/ml. The daily variations during maintenance

Table V *Relationship between T/2–serum concentration at withdrawal–total body store–"proper maintenance dose" Fall in serum concentration after withdrawal for 1–3 days*

For calculations see text

	Corresponding serum concentration				
T/2 (d)	At withdrawal ng/ml (100 %)	After 24 h ng/ml (%)	After 72 hours ng/ml (%)	Total body store (mg)	Proper maintenance dose" (mg/day)
2.0	2.4	1.7 (71)	0.9 (35)	0.34	0.35
2.4	3.9	2.9 (76)	1.6 (44)	0.40	0.30
2.7	5.0	3.9 (77)	2.3 (47)	0.44	0.27
4.1	10.0	8.4 (84)	6.0 (60)	0.64	0.19
5.5	15.0	13.2 (88)	10.3 (69)	0.84	0.14
6.9	20.0	18.1 (90)	14.8 (74)	1.04	0.12
8.3	25.0	23.0 (92)	19.4 (78)	1.24	0.10
9.7	30.0	27.9 (93)	24.2 (81)	1.44	0.08
11.0	35.0	32.9 (94)	29.0 (83)	1.64	0.07
12.4	40.0	37.8 (95)	33.8 (85)	1.85	0.06
14.0	45.6	43.2 (95)	39.2 (86)	2.07	0.05

therapy can also be calculated from the observed results of $T/2$ and mean serum concentration (see below)

Table V column 3 demonstrates that these variations usually are about 10%. With a short $T/2$ the variations may increase to 30%. However this is accompanied by low serum concentrations, and the variations in ng/ml are rather small. If we want to decrease such daily variations, we can divide the maintenance dose into two daily doses. Lukas & Peterson (17) state that the daily variation is about 20–30% but this is above our observed and calculated mean variation.

All these results and calculations are concerned with digitoxin concentration in serum. The variations in myocardial concentration probably follow serum concentration, but the rate of equilibration between myocardium and serum is not known. Digoxin experiments in dogs indicate that the biological half life is the same for serum and myocardium (4)

Correlation serum digitoxin – creatinine – weight – age (Table I)

This report shows no significant correlation between serum concentration and age. The common experience that elderly patients should have smaller oral doses can be explained by increased myocardial sensitivity in old age.

The correlation between serum concentration and weight is small. For instance, the calculated mean serum concentration for a 45 kg patient is 20.2 ng/ml, while the 90 kg patient using the same oral dose has a mean concentration of 15.4 ng/ml. Other authors have found a more significant correlation. According to Lukas & Peterson the above values should be:

45 kg – 20.2 ng/ml, 90 kg – 11.1 ng ml.

No correlation was found between serum concentration and creatinine (see below under $T/2$ correlations)

Loading – no loading doses

Figs. 2–6 demonstrate the effects of repeated small doses as compared to the whole loading portion in a single dose. Fig. 2 shows that with 0.1 mg each day we obtained / maximal serum concentrations after 7 days and / maximal values after 14 days. When the heart failure is moderate this is an easy and safe prescription, and clinical control for possible intoxication should be made after treatment for 2 weeks. The observed serum concentrations of Fig. 2 are below the theoretical values based on $T/2 = 6.855$ days (8). However the number of patients in this group was small. This gives a somewhat unreliable mean concentration, and the results are within the theoretical value ± 2 S.D.

On loading doses the patient' total body stores are saturated in few days. With the largest

loading doses therapeutic serum concentrations are seen after an hour but myocardial saturation occurs at a slower rate as observed by the maximal inotropic effect after 4–12 hours (9)

Serum half life

After withdrawal of maintenance therapy the mean serum half life was 6.9 ± 2.67 days. This is in agreement with the few observations by other authors (Table III)

The determination of $T/2$ has only been possible in a group of patients using 0.1 mg digitoxin. However the $T/2$ for the other groups can be estimated according to equation 2.

Looking at Table I we observe that weight and age are much the same for the different groups. Some of these differences are significant, but the calculations of correlations have shown that such differences give very small deviations in serum concentration. Accordingly we can put $V \approx V'$ The observed differences in mean serum concentrations must be chiefly due to different dose absorption, elimination. In order to estimate the variations in $T/2$ we must disregard our suspicion of variations in absorption and assume that F is the same in all 4 groups. Equation 2 is solved for the unknown $T/2$ and the results are: 0.071 mg maintenance dose – 8.5 days, 0.05 mg – 8.4 days, 0.036 mg – 11.4 days.

The validity of these calculations may be questioned, and the results can be interpreted in two ways. To begin with, patients in the low-dose groups really have increased $T/2$ as estimated. Secondly the absorption may be different, with a lower fraction of absorption in the high-dose group. This last possibility cannot be verified by the present study In either case the adjustment of doses as initiated by the general practitioner has been clearly necessary

Table II shows a high correlation between $T/2$ and serum concentration at withdrawal. This means that a high concentration is partly the result of long $T/2$. Further the correlation between $T/2$ and serum creatinine was good. This shows that renal function is the chief determinant of $T/2$. The correlation between $T/2$ and age probably reflects the well known correlation between age and renal function even if serum creatinine remains within the accepted normal range in old age. (Regression line in this study: creatinine = 0.43 + 0.01 years, $p < 0.001$ $r = 0.72$.) As expected, there was no correlation between $T/2$ and weight.

The lack of correlation in steady state serum concentration – creatinine compared with the significant correlation $T/2$–creatinine may seem somewhat conflicting. The explanation is that serum concentration during maintenance therapy is the final result of absorption, distribution, metabolism and elimination. Variations in the first three factors, which at present are rather unknown, tend to eliminate correlations which are evident when we study only one factor $T/2$ creatinine. Further other ways of elimination, for instance enterohepatic circulation with digitoxin loss in faeces, will modify the correlation serum concentration – creatinine.

Based on our results of $T/2$ and maintenance dose other calculations can be made (8, 14, 18). In the steady state the elimination of digitoxin and metabolites is a linear function of the oral dose, and after withdrawal the amount remaining in the body decreases by an apparent first-order exponential decay:

$$X = X_0 \cdot e^{-kt} \quad (3)$$

(X = total amount of digitoxin in the body t days after withdrawal, X_0 = total amount of digitoxin in the body at start of withdrawal, e = base of natural logarithms, $k = \ln 2/(T/2)$ = rate constant for elimination.) The daily maintenance dose D is the difference between X and X_0 when t = 1 day:

$$D = X_0(1 - e^{-k}) \quad (4)$$

Based on the calculation of the regression line and assuming a constant maintenance dose of 0.1 mg the relationship between $T/2$ and serum concentration is presented in Table V columns 1 and 2. After withdrawal for 1–3 days the decline in serum concentration is demonstrated in columns 3 and 4

Changes in $T/2$ also lead to large variations in the accumulation of digitoxin in the body. Total body stores of digitoxin after 0.1 mg each day and with different $T/2$ are calculated according to equation 4 and presented in column 5

If we maintain a constant dose of 0.1 mg each day deviations in $T/2$ from mean value must result in many undertreated or intoxicated patients. Our aim must be to adjust the maintenance dose in such an order that serum con-

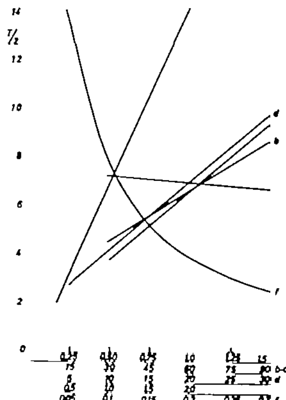


Fig. 7 Correlation $T/2$. — serum creatinine (mg/100 ml), b = age, — weight, d = serum digitoxin at withdrawal (ng/ml). The calculated relationship between $T/2$ and (total body store of digitoxin in mg) and f ("proper maintenance dose in mg/day) are demonstrated.

centration and total body stores are kept in an optimal range. This can also be calculated from equation 4

The optimal values of serum concentration and body stores are not known. If we use the mean serum concentration 18 ng/ml for the 81 patients in the 0.1 mg group and mean $T/2 = 6.855$ days, the total amount of digitoxin in the body would be 1.040 mg. In most of our patients the dose has been regulated by the general practitioner without knowledge of serum concentration. Many of the patients in the 0 1 mg group had a rather low serum concentration, and the dose could safely be increased. We have rather few true intoxications and our doses are generally smaller than reported from other countries. Average maintenance dose of digitoxin has often been stated to be 0.15 mg. Further experience indicates that the average loading dose 1.2 mg is correct instead of 1.040 mg. If we accept this value as a proper loading dose and optimal value for total digitoxin stores, the proper maintenance dose should be 0 115 mg for a patient with $T/2 = 6.855$ days. The proper maintenance doses" for patients with other serum half lives are presented in Table V column 6 and in Fig. 7

Fig. 7 should be regarded as a guideline for the individualization of digitoxin medication. In the particular patient the serum concentration in the steady state demonstrates his ability to absorb, distribute metabolize and eliminate digitoxin. After withdrawal of digitoxin for 5–7 days we can determine his rate of elimination From these results we can calculate his total body store before withdrawal and the proper maintenance dose If he has a deficit in total body store this is replaced by an extra dose and the new maintenance dose is initiated. After about 5 half lives a new steady state is obtained, and a serum determination is done to verify that the patient behaves according to our calculations. In some cases it may be inconvenient to withdraw digitoxin for a week, and the patient may rather need an increase in dose. A tentative new maintenance dose is selected, and the increase in serum con centration is followed by regular determinations. The time interval for obtaining a new steady state makes possible the calculation of k and $T/2$ (8)

This tentative approach should be used for individualization of digitoxin therapy According to our present laboratory and clinical experience we would advise adjustment of the serum concentration in the upper normal range above 18 ng/ml. Values above 30 ng ml may be indicated in patients with serious heart failure if they can tolerate medication. Such patients should be controlled at regular intervals for intoxications and for disturbances which may change the myocardial sensitivity and the rate of elimination. When the clinical situation is stable the serum concentration shows small deviations during maintenance therapy

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GLUCOSE TOLERANCE, PLASMA INSULIN AND LIPIDS IN RELATION TO ADIPOSE TISSUE CELLULARITY IN MEN AFTER MYOCARDIAL INFARCTION

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Abstract. Glucose and insulin values in plasma after peroral glucose tolerance test, triglycerides and cholesterol in plasma, body composition and adipose tissue fat cell diameter were determined in nonselected men surviving myocardial infarction and were compared with the same variables in randomly selected men from the same region of equivalent age. The patients with myocardial infarction ere characterized by diminished glucose tolerance and elevated plasma lipids when compared with controls. The diminished glucose tolerance was more pronounced in patients with signs suggesting severe myocardial damage or complications to the myocardial infarction. Body fat, fat cell size or insulin values during glucose tolerance test were, however not increased in patients with myocardial infarction. Fat cell size and fasting insulin or the sum of plasma insulin during the glucose tolerance test cor related positively in both groups of older men. Plasma triglycerides showed significant, positive correlations with the sum f insulin and glucose values during the glucose tolerance test. Patients ith endogenous hypertriglycerid-emia had higher insulin and blood glucose values and more body fat than patients without plasma lipid ab-normalities. It was suggested that increased plasma tri-glycerides in patients with myocardial infarction are com-bined with decreased glucose tolerance and an increased insulin secretion associated with enlargement of adipose tissue fat cells.

The nature of the metabolic aberration often seen in young patients who have suffered a myocardial infarction is complex. In addition to increased plasma lipid levels and a decreased glucose toler ance elevated plasma insulin and body weight have also been reported (1 2, 3 14 16 17 18 21 22, 25 26, 34 35 40 41 45) An association between the latter two variables is well known (24 32, 37 38) the size of adipose tissue fat cells apparently being a stronger determinant of plasma insulin concentration than the amount of body fat (5) Plasma insulin, in turn, is of importance for the production of hypertriglyceridemia (1 17 18 41 44) It was therefore thought to be of in terest to perform fat cell size measurements in a group of young men with myocardial infarction and to relate these results to metabolic variables. The aim of the present work was thus to apply a new technique and new approach to this frequent ly studied problem, viz.

1 To try to elucidate which metabolic ab-normalities characterize young, non-selected pa-tients after a myocardial infarction by performing comparisons with a randomly selected material of the same sex and equivalent age from the same region.

2. To study the association of f t cell size with the metabolic aberrations found in ischemic heart disease.

MATERIAL

The city of Gothenburg is situated on the est coast of Sweden and has about 400 000 inhabitants. Admission and follow-up of all patients ith myocardial infarction before the age of 55 have since 1968 been organized by Myocardial Infarction Clinic In this ay uniform treat-ment and follow-up f these patients are ensured. One hundred and seven men ith myocardial infarction during the period Jan. 1st 1968 to May 31st 1969 survived 3 months after their first myocardial infarction. Every sec ond patient was selected randomly for physical training 3 months after the myocardial infarction. The results in this group will be presented in separate publication Three of the 54 non-training patients had overt diabetes mellitus and were excluded

The patients with myocardial infarction ho had one

Table I *Results of determinations in myocardial infarction patients with or without a large infarction or complications (means ± S E.M.)*

Myocardial infarction group		Glucose values (mg/100 ml) 0	30	60	90	120	Sum	Insulin values (μU/ml) 0	30	60	90	120'	Sum
Without complications	62	73 ±2	124 ±4	119 ±5	100 ±5	86 ±3	500 ±15	13 ±1	87 ±9	118 ±11	122 ±16	97 ±13	436 41
With complications	17	76 ±4	142 ±8	158 ±15	156 ±17	136 ±17	596 ±56	13 ±1	101 ±34	102 ±31	122 ±38	119 ±35	457 [illegible]

of the following signs or symptoms in connection with the myocardial infarction and at an examination 3 months thereafter were analyzed separately: pronounced increase of SGOT (> 200 U), cardiac arrythmia, signs of pulmonary congestion, or heart size increase at heart lung X-ray or shock. There were 17 such patients in the whole sample of 107 patients and 8 in the non-training half of this sample. Since they were shown to differ metabolically from patients without such complications (Table II) they were also excluded from the main group of patients who were compared with controls in the present work, and this remaining group thus consisted of 43 men. Their mean age was 49.4 years (range 37–55).

The myocardial infarction patients were compared with 81 randomly selected 55-year-old men from the men born 1913 study (43). Five men had suffered myocardial infarction or had angina pectoris and were therefore excluded from the present investigation. This material has previously been presented in detail (5).

METHODS

When discharged from hospital after the myocardial infarction the patients were given similar instructions. Moderate physical activity was prescribed in the form of short walks and the patients were recommended to avoid bed rest. Obvious dietary abnormalities were corrected by a dietician's advice towards a diet similar to an average Swedish diet (11). Seventeen of the patients with myocardial infarction were on medication at the examination 12 months later. The drugs and number of men taking the drug (within parentheses number at 3 months) were: nitroglycerin, 8 (4) men, digitalis, 3 (2) men, potassium chloride, 2 (1) man, dicoumarol, 4 (4) men, tranquillizers, 6 (6) men.

The examinations were performed 3 and 12 months after the myocardial infarction. The patients were instructed to report to the laboratory without too much physical activity in a fasting state and not having smoked for 12 hours before the investigation. Venous blood was drawn into chilled heparinized tubes for determination of blood glucose (28), plasma insulin (20), cholesterol (15), triglyceride (13), and paper electrophoresis of plasma lipoproteins (27). After peroral intake of 100 g glucose, blood glucose and plasma insulin were determined at 30, 60, 90 and 120 min by repeated vein-punctures with the patient resting.

On another day body composition was determined by isotope dilution methods by measuring exchangeable potassium and total body water (30, 31), whereafter body fat could be calculated (33).

Table II. *Results of determinations in different groups (means ± S E.M.)*

Group		Glucose values (mg/100 ml) 0	30	60	90	120'	Sum	Insulin values (μU/ml) 0	30'	60	90'	120'	Sum
Myocardial infarction, 3 months	46	72 ±2	126 ±4	121 ±7	108 ±7	93 +6	511 ±21	12 ±1	90 ±10	102 ±12	111 ±17	93 ±13	412 17
Myocardial infarction 12 months	24	73 ±3	127 ±7	124 ±9	111 ±9	94 ±10	525 ±34	12 ±3	58 ±7	82 ±11	104 ±26	90 ±27	348 64
Controls, 55-year-old	76	64 ±1	121 ±3	108 ±5	86 ±4	69 ±3	448 ±14	10 ±1	79 ±6	95 ±9	96 ±1	55 ±6	337 ±29
Comparison between differences in the same pats. at 3 and 12 months	26	s.	s.	n.s.	n.s.	s.	n.s.	s.	p 0.01	p<0.01		p 0.05	p [illegible]

ody fat (kg)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)
±1	274±6	149±7
±2	285±14	156±15

Fat cell diameter was determined on 21 of the infarction patients in percutaneous adipose tissue biopsy from the abdominal wall as described previously (5). Average fat cell volume and weight were determined as described by Goldrick (19).

The controls were investigated similarly as also described previously (5). Body fat was, however obtained from anthropometric measurements and regression equations obtained from other subsamples of the same population (5). Fat cell size was determined in 49 men.

From the total population of patients examined 3 months after their myocardial infarction (107 men) a group described as endogenous hypertriglyceridemia was selected. This group (26 men) was defined as having plasma triglyceride value above 150 mg/100 ml plasma, distinct pre-β-lipoprotein band on paper electrophoresis, and cholesterol below plus one S.D. of the controls. Another group had both triglyceride and cholesterol values below plus one S.D of the controls and no pre-β-lipoprotein band on paper electrophoresis. This group (37 men) was called infarction patients without plasma lipid abnormality

RESULTS

Table I shows the results of the analysis of the different groups of myocardial infarction after

y fat (kg)	Fat cell size (μg)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)
15±1	96±3[a]	279±10	150±8
13±2	96±3	266±7	140±15
16±1	102±2[b]	257±5	109±7

−21 [b] 49

subdivision according to the degree of severity of their myocardial infarction and the clinical state at the 3 months follow-up. It is apparent that the infarction patients with signs of severe myocardial damage or complications to their myocardial infarction had a more pronounced diminution of the glucose tolerance than patients without complications (higher glucose values at 60, 90 and 120 min, $p < 0.05$ or lower) The patients with complications were therefore excluded from comparison with controls in the sequel.

The examinations were repeated 12 months after myocardial infarction in 24 of the patients without complications. As seen in Table II, plasma insulin values were lower at the last examination, while the other values did not differ

Table II also shows that the infarction patients had a decreased glucose tolerance in comparison with controls (higher glucose values at 0, 90 and 120 min and higher sum of glucose values at 3 or 12 months, $p < 0.02$ or lower) As far as plasma insulin is concerned, the infarction patients showed a higher value at 120 min ($p < 0.02$) at the first examination, but the controls had a higher value at 30 min ($p < 0.05$) at 12 months. The other values did not differ

Both plasma cholesterol and triglyceride were higher in the group of myocardial infarction patients than in the control group ($p < 0.02$ or lower) at 3 months. At 12 months only the triglyceride value was higher ($p < 0.02$) Body fat and fat cell size did not differ

Figs. 1 and 2 show the positive correlations between fat cell size and fasting insulin or sum of insulin values during the glucose tolerance test for middle-aged controls and myocardial infarction patients. Fat cell number did not correlate with plasma insulin values.

Sum of insulin and sum of glucose values during the glucose load, on the one hand, and plasma triglycerides on the other correlated in the controls (correlation coefficients 0.30 and 0.25 $p <$ 0.01 and < 0.05) and in patients with myocardial infarction (correlation coefficients 0.36 and 0.26, $p < 0.01$ and < 0.05) Fat cell size did not correlate significantly with plasma triglycerides or with the sum of glucose values during the glucose tolerance test.

Positive correlations were thus found between plasma triglycerides, on the one hand, and insulin and glucose values on the other A further

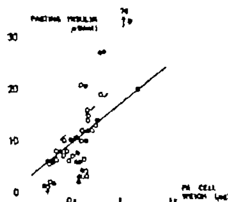


Fig. 1. The relationship between adipose tissue fat cell weight and plasma fasting insulin in middle-aged men with and without myocardial infarction.

	Regression eq.	n	r	p(r≠0)
Controls (O ——)	$y = 16 + 1.6$	49	0.37	< 0.01
Myocardial infarction (● – –)	$y = 20x + 2.7$	21	0.45	< 0.05

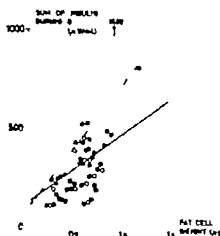


Fig. The relationship between adipose tissue fat cell weight and sum of plasma insulin values during glucose tolerance test in middle-aged men with and without myocardial infarction. Symbols as in Fig. 1

	Regression eq.	n	r	p(r)
Controls	$y = 356x + 121$	49	0.36	0.02
Myocardial infarction	$y = 654x + 83$	1	0.64	< 0.01

analysis of these interrelations was made by comparing infarction patients with endogenous hypertriglyceridemia with patients without plasma lipid abnormality. Table III shows that the patients with endogenous hypertriglyceridemia had not only an elevated sum of both glucose and insulin values during glucose tolerance test and a trend higher fasting glucose and insulin values, but more body fat.

DISCUSSION

In comparison with controls of equivalent age the myocardial infarction patients were characterized by a decreased glucose tolerance and by elevated plasma lipids. These are well-known findings (14 21 22, 26, 35 40 44 45)

The decreased glucose tolerance was more pronounced in a group of patients with signs of severe myocardial damage. The degree of severity of the myocardial infarction and sequelae to it thus probably interfere with glucose tolerance. This has also recently been found by Paasikivi (36). These men were excluded from comparisons with controls because they are likely to have had an activated sympathetic nervous system at the 3 months follow-up (23)

One might question whether the decreased glucose tolerance is a consequence of the episode of myocardial infarction or a metabolic characteristic of these patients also before their myocardial infarction. Ostrander et al. (35) found in a prospective study that glucose intolerance is an

Table III. *Metabolic variables and body fat in myocardial infarction groups (means ± S.E.M.)*

Myocardial infarction group		Glucose (mg/100 ml) Fasting	Glucose (mg/100 ml) Sum	Insulin (µU/ml) Fasting	Insulin (µU/ml) Sum	Triglyceride (mg/100 ml)	Cholesterol (mg/100 ml)	Body fat (kg)
Without plasma lipid abnormality	37	70 ± 3	506 ± 34	10 ± 1	322 ± 35	105 ± 5	250 ± 5	13.2 ± 0.8
With hyperprebetalipoproteinemia	26	77 ± 3	596 ± 35	14 ± 2	500 71	193 ± 8	260 ± 6	17.6 ± 1.7
p		< 0.1	< 0.05	< 0.1	0.01	0.001	n.s.	< 0.01

important risk factor for coronary artery disease. Furthermore, patients without lipid abnormality had the same glucose tolerance as controls (cf Tables II and III). This does not speak in favour of a decreased glucose tolerance as merely a secondary consequence of the myocardial infarction episode.

When infarction patients and controls of the same age were compared, plasma insulin during glucose tolerance test differed only in a single point, and body fat and fat cell size did not differ. An increased insulin level in plasma, fat ness or expanded fat cells are thus apparently not characteristic of a nonselected group of patients with myocardial infarction. Fat cell size and plasma insulin showed rather strong positive correlations in the control group, as previously described (5). This was also the case in myocardial infarction patients. Increased plasma insulin is thus found in subjects with or without myocardial infarction when they have expanded fat cells.

There were correlations between the sum of insulin values and the sum of glucose values during glucose tolerance test, on the one hand, and the plasma triglycerides on the other. A group of endogenous hypertriglyceridemia patients—type IV according to Levy and Fredrickson (29)—was shown to have not only decreased glucose tolerance but also increased plasma insulin and body fat in comparison with infarction patients without plasma lipid abnormality. As shown before (1, 17, 18, 41, 44) this demonstrates the association between plasma triglyceride elevation, on the one hand, and plasma insulin in combination with a decreased glucose tolerance on the other also within a population of infarction patients. Due to the limited number of patients who were subjected to fat cell size determinations a direct analysis of this factor could not be performed in these different subgroups. The positive correlations between fat cell size and insulin show, however, that when high insulin levels are present, there is also an increase in fat cell size. This has also recently been found within a group of obese (10) as well as in a group of myotonic dystrophy patients (9).

The cause-effect relationship between fat cell size and plasma insulin is not known, although some information is available. It does not seem likely that insulin insensitivity of large fat cells (42) could explain an elevation of plasma insulin, because the uptake of glucose in adipose tissue is too small to create a compensatory hyperinsulinemia if blocked (4, 8). It is more likely that hyperinsulinemia is caused by insulin resistance in other tissues such as muscle, shown to be insulin-insensitive in obesity with presumably enlarged fat cells (12, 39), because physical training of obese patients with maintained fat cell size normalizes hyperinsulinemia (6). It therefore seems probable that fat cell enlargement is secondary to hyperinsulinemia. This is supported by the observation that glucose uptake in fat cell triglyceride is proportional to fat cell size and to plasma insulin (4). Triglyceride synthesis from glucose in vitro is also proportional to fat cell size (7). An increased synthesis of triglycerides in the liver, causing endogenous hypertriglyceridemia as suggested by Reaven et al. (41), would be an analogous situation in the liver to that causing enlarged fat cells by increased triglyceride synthesis in adipose tissue. Deficient mechanisms of removal of plasma triglycerides is an alternative explanation.

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ALPRENOLOL AS MAINTENANCE THERAPY FOR THE PROTECTION AGAINST ARRHYTHMIA RELAPSE IN PATIENTS WITH DC-CONVERTED CHRONIC ATRIAL FIBRILLATION

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Abstract. There is a constant search for new drugs, free from side-effects, to maintain sinus rhythm following DC conversion of atrial fibrillation. Adrenergic β-receptor blockers have been tried with rather disappointing results, possibly due to the relatively low dosage used. The present trial was performed in order to evaluate the efficacy of a dose almost twice the one earlier used. By incrementing the dose, alprenolol 100 mg q.i.d. was given to 66 patients and 75 mg q.i.d. to two others. No patient converted spontaneously on alprenolol. After DC-conversion 80% of the patients reverted to sinus rhythm. Further follow-up showed a relapse rate of 17% after 24 hours, 65% after one month, 79% after three months, and 85% after six months. In comparison with previously reported studies, these figures are all higher than usual. Possible reasons for this are discussed. The incidence of post-conversion arrhythmias remained low despite uninterrupted digitalis therapy. Sinus node depression following DC-conversion, as has been reported in propranolol studies, did not occur and in our experience it is not necessary to discontinue alprenolol prior to DC-conversion. Ten patients who relapsed were subsequently treated with a combination of alprenolol and quinidine, but the results were disappointing. In a few cases treatment was stopped with the onset of bradycardia due to sinus node depression. Although generally adrenergic β-receptor blockade appears ineffective in preventing relapse following DC-conversion, it should be considered in selected cases where it may prove to be a valuable alternative, as illustrated by one of the treated patients.

After the introduction into clinical practice by Lown et al. 1961 (16) of electroconversion of atrial fibrillation by means of a direct current shock (DC-conversion) this method has subsequently proved to be both safe and effective (19). The maintenance of the acquired sinus rhythm, however, offers greater problems, and various anti-arrhythmic drugs have been used in order to diminish the relapse risk. There is a constant search for more effective drugs with fewer side effects. Among the newer ones tried in recent years are the adrenergic β-receptor blockers (9, 23, 25). The rather disappointing results in these studies may be due to too low a dosage. The present trial was undertaken in order to evaluate the efficacy of a dose roughly twice that of the studies cited, using alprenolol (Aptin®) a specific adrenergic β-receptor blocker (30).

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MATERIAL AND METHODS

Patients with chronic atrial fibrillation have been collected from the wards of internal medicine in five hospitals. Uniformity of selection and treatment procedure was achieved by the use of a standardized sorting-card (Fig. 1). These were double with carbon paper between. One copy was always retained at the hospital and the sorting-cards were collected centrally. The symptoms listed on the right were asked for specifically every time a card was filled in, which was 1) when alprenolol treatment started, 2) at discharge from hospital after DC-conversion, and 3) at follow-ups one, three and six months after conversion. Patients with manifest cardiac decompensation, postural hypotension, bronchial asthma or chronic bronchitis with considerably reduced ventilatory capacity were excluded.

Laboratory blood sample tests relevant to liver, renal and blood formation function were performed before and occasionally during alprenolol treatment. The serum electrolytes were also checked for normality before DC conversion. All patients had a routine physical examination, chest X-ray and ECG before starting alprenolol, which was instituted at a dose of 25 mg q.i.d. and increased to a final dose of 100 mg q.i.d. whenever possible. The final dose was attained either in one week in hospitalized patients (75% of the cases) or in three weeks in ambulatory patients.

SORTING-CARD ALPRENOLOL

No Dat

Name Trial No. Hospital
Date of birth Sex Physician
Diagnosis Height Subjective complaint
Previous therapy Weight
Duration I AF years months Hb General condition
Physical signs General condition Reticulocytes Heart failure
Heart Leukocytes Resp distress
Lungs Platelets Diarrhea
Other sign Creatinine Constipation
Bilirubine Nausea
Heart siz ml ml/BSA Alk phosph. Vomiting
ECG SGOT Dizziness
SGPT Headache
Other drugs 1 Since Glucose Dysuria
2 U Protein) Mood
3 Glucose) Intercurr disease
Alprenolol dosage Sediment AF relapse (circumstances)
DC shock dat Wsecs Blood diff count
Remark

Remarks

No of t blet returned Dosage taken
— — give out Next control
) By X-ray in two planes m) Albestur) Cinnetix

Fig. 1. The standardized sorting-card which was used for the collection of data.

All patients were hospitalized for DC-conversion and were pre-treated with anticoagulants for 3–4 weeks in most cases. Digitalis was not discontinued before conversion in digitalized patients. The DC-conversion was performed after the final dose of alprenolol had been taken for tw days, and 1 3 hours after the last dose. In patients in whom sinus rhythm was attained by the electric shock, the alprenolol treatment was continued.

The conversion was performed with synchronized direct current defibrillator. The electric energy was increased stepwise from 100 Wsecs to 300–400 Wsecs. As premedication 50 mg pethidine was given. During DC-conversion light intra enous barbiturate anaesthesia was used. Heart action immediately before nd fter the conversion was monitored continuously on an oscilloscope. In addition, a complete ECG recording was made before and immediately after defibrillation.

Sixty-eight patients ere collected for the study. One had to be excluded because of tablet failure (cerebral embolus shortly after conversion made the patient unable to swallow the tablets), and another was excluded due to development of dyspnoea and oedema during the (ambulant) alprenolol run-in period. Of the remaining 66 patients, 45 ere men and 1 women. Mean age as 59.9 years (range 31 79). The origin of atrial fibrillation was judged to be rheumatic heart disease predominantly with mitral valve involvement, in 11 cases (17%), arteriosclerotic heart disease in 49 cases (74%), hyperthyreosis (treated) in 1 case, and "lone atrial fibrillation in 5 cases (8%). Data such as duration of trial fibrillation, heart size, digitalis therapy and previous DC-conversion attempts are given in Table I.

1 ten patients (at two of the hospitals) reverting to atrial fibrillation within one month afte conversion, a new conversion attempt was made after combined pretreatment with alprenolol and quinidine. Seven patients received alprenolol 50 mg q.i.d. plus quinidine in sustained release form (Kinidin Duretter®, also known as Kinidin Durules®, Kinidin Duriles®, Chinidin Durules®, Chinidin Durules®) corresponding to 0.4 g monosulphate twice daily. Three patients received alprenolol 100 mg q.i.d. plus quinidine 0.4–0.8 g twice daily.

RESULTS

A final dose of alprenolol (400 mg daily) was attained by all patients except two, who stopped at 75 mg q.i.d. (see below). None converted spontaneously on alprenolol. Of the 67 converted patients 53 (80%) developed sinus rhythm. One of these patients was excluded due to tablet failure (see above). Of the remaining 52, 9 (17%) had relapsed to atrial fibrillation after 24 hours. At the one-month follow-up 34 patients (65%) had relapsed. After three months 41 (79%) and after six months 44 (85%) patients had relapsed.

At the one-month follow-up five patients turned

Table I. *Pertinent data for the 66 patients followed up*

	Total no. of pats.	No. of pats. in sinus rhythm after			
		0 h	24 h	3 mo.	6 mo
Duration of atrial fibrillation					
Less than one year	30	25	21	7	6
More than one year	35	27	22	4	2
Unknown	1	—	—	—	—
Heart volume					
Normal[a]	14	12	9	4	2
Increased by less than 200 ml	38	29	25	5	4
Increased by more than 200 ml	12	9	7	2	2
No information available	2	2	2	—	—
Digitalis therapy					
Yes	57	46	38	9	6
N	9	6	5	2	2
Previous DC-conversion					
Yes	19	17	13	3	1
No	47	35	30	8	7

[a] Males < 450 ml/m² BSA, females < 400 ml/m² BSA.

out not to have taken the prescribed dose of alprenolol. Four of these had reverted to atrial fibrillation. For five of the patients who were still in sinus rhythm at three months, a lower dose (200 mg daily in one case 300 mg daily) was prescribed from then on by the physician. Four of these patients still maintained sinus rhythm at the six-month control.

Of the 14 patients who did not revert to sinus rhythm at conversion, 9 were given up to 400 Wsecs and four up to 300 Wsecs. In one patient no further conversion attempt was made after 200 Wsecs, when an arrhythmia occurred (see below). Age, sex, diagnosis, duration of atrial fibrillation, digitalis therapy or previous DC-conversion do not seem to have influenced the immediate conversion result. The material is too small to permit evaluation of the corresponding results at three and six months follow-up as can be seen in Table I.

Arrhythmias immediately after DC-conversion were noted in some cases: ventricular extrasystoles in four patients (6%), and supraventricular extrasystoles in six patients (9%). One patient had a few short runs of ventricular tachycardia. Brady arrhythmias occurred in five patients (8%). One had intermittent sino-auricular block, two had sinus bradycardia interrupted by nodal rhythm. One patient had a pure sinus bradycardia. The fifth patient had asystolia after 200 Wsecs, following by first a ventricular then a supraventricular arrhythmia, subsequently relapsing to atrial fibrillation. This patient also had a high digitalis maintenance dose. All arrhythmias disappeared spontaneously and required no specific therapy.

Among ten patients re-converted after combined pre-treatment with alprenolol and quinidine three of the seven treated with the low-dose combination acquired sinus rhythm spontaneously. Only two of these seven patients remained in sinus rhythm after one month. No arrhythmias were observed in these patients. The three patients treated with the high-dose combination all had signs of sinus node depression with bradycardia, nodal rhythm (as well as subjective side-effects—nausea, dizziness) at quinidine dosages of 1.2 g daily or more, and treatment was subsequently discontinued with eventual return of atrial fibrillation.

Side-effects

Two patients did not tolerate more than 300 mg alprenolol daily one due to heartburn and the other because of dizziness on 400 mg daily. One patient developed dyspnoea and oedema during the run-in period on a dose of 300 mg alprenolol daily. She was not digitalized. The insufficiency was successfully treated with digitalis and diuretics after withdrawal of alprenolol. Another (digitalized) patient developed cardiac decompensation after five months' treatment in the course of an acute nephritis following a streptococcal peritonsillitis. The role of alprenolol (300 mg daily from the beginning) was considered doubtful, but the drug was discontinued in the course of treatment of the decompensation. Shortly afterwards artial fibrillation recurred. Of the symptoms specifically noted on the data chart it may be noted that dyspnoea and/or oedema was recorded in 11 patients before cardioversion, but only in 4 patients at discharge from hospital. At subsequent follow-ups these symptoms were noted in four other patients, all having relapsed to atrial fibrillation.

Other symptoms listed occurred occasionally but did not cause appreciable trouble of the patients or discontinuation of the therapy. The

laboratory tests, which were checked at least once during the alprenolol treatment in each patient, did not show appreciable deviations suggesting undue toxic influence of the drug.

DISCUSSION

Quinidine is the most commonly used anti arrhythmic agent for maintenance treatment of sinus rhythm after DC-conversion of chronic atrial fibrillation. Earlier it has been maintained that a prophylactic effect is uncertain (3 7 8, 18) In other studies from recent years it appears that the relapse rate is diminished even at long term follow-ups (4 5 11 12). Even in the studies showing the best results, however about 40% of the patients relapse to atrial fibrillation within one year (4 17) Procainamide has also been used and Szekely et al. (23) for instance, have reported results comparable to those of quinidine. Side-effects and complications of the drugs must also be taken into consideration. The most serious is probably ventricular fibrillation caused by quinidine. One should be able to avoid this by an individual dosage regimen, control of serum levels, and by not giving quinidine before DC-conversion (3 27) Gastrointestinal allergic and haematologic complications make patients intolerable to quinine in some cases (5) Procainamide has been shown to increase the risk of lupus syndrome and a positive LE cell phenomenon to an extent previously not suspected (1). Hence the search for a new effective drug, with less side-effects, to prevent relapse to chronic atrial fibrillation is desirable.

Results from studies with adrenergic β receptor blocking drugs have previously been reported by Tsolakas et al. (25) and Szekely et al. (23) who used propranolol in the dose of 60–80 mg daily No prophylactic effect greater than without treatment could be seen in these groups of 18 patients each. Hillestad and Andersen (9) administered alprenolol 160–200 mg daily to 98 patients, 67% of whom were converted by DC-shock. Three months after conversion 64% of the converted patients had relapsed to atrial fibrillation. The same authors made cross-over studies with repeated conversion attempts in patients who relapsed, and found quinidine to be more effective than alprenolol in preventing relapse to atrial fibrillation. They considered this somewhat surprising in view of the similarities between quinidine and the β-receptor blocking drugs. As an explanation they propose that the vagolytic action of quinidine is beneficial, as vagal stimulation increases the possibilities of arrhythmia relapse to atrial fibrillation (2) Adrenergic β-receptor blockade would indirectly have an opposite action by unmasking the vagal stimulation through the inhibition of the sympathetic stimulation.

The dosage of adrenergic β-receptor blockers is fairly low in the three reported studies. Experimentally it can be shown that an appreciable inhibition of the adrenergic β-receptor response is present already at the dose level used in these studies (propranolol 20 mg single dose, alprenolol 50 mg single dose) whereas the direct membrane effect (the quinidine-like effect) does not have an appreciable anti-arrhythmic and haemodynamic effect until at higher dose levels (30). It has also been shown that, at least for the treatment of some arrhythmias, this direct membrane effect seems to be necessary (6, 26) A possible explanation of the earlier results with adrenergic β-blockers could therefore be that the dosage was too low

The immediate success ratio for DC-conversion is between 80 and 90% in various studies and not below 70% after 24 hours (3 14 17 18, 23, 29). The immediate success ratio in our study was 80% and 61% at 24 hours after conversion. Thus the relapse rate seems to be slightly higher than usual already one day after conversion. After three months 42–69% of converted patients are reported to maintain sinus rhythm when treated with quinidine (3 4 11 17 23 29). The corresponding figure in our study is 21% and after 6 months only 15% of the patients remain in sinus rhythm. In the study in which alprenolol was used in a lower dose (9) there was a better result at three months follow-up with 36% of the patients still remaining in sinus rhythm.

Compared to most other studies our material contains a larger proportion of patients in whom the atrial fibrillation was considered to be due to arteriosclerotic heart disease. However it has been stated in different studies that the long-term results in this type of patients do not differ from the results in patients with rheumatic valve disease, which is usually the dominating diagnosis (3 5 12, 29) Hence this is probably not the explanation of the poor long-term results. On the

other hand the immediate conversion result in patients with arteriosclerotic heart disease seems to be poorer than in patients with rheumatic valve disease, and conclusions cannot therefore be drawn from our study concerning the effect of adrenergic β-receptor blockade for the immediate result of DC-conversion. Occasional cases have been reported in which DC-conversion did not succeed until after pre-treatment with adrenergic β-receptor blockers (6).

The occurrence of previous conversion attempts and subsequent maintenance therapy (quinidine or procainamide) did not influence the results, as the relapse rate in previously uncon erted patients is high (72% at three months). Thus treatment with alprenolol has not been a better alternative except possibly in a few cases. The pa tient who developed cardiac insufficiency after peritonsillitis had had several previous DC-conversions with different anti-arrhythmic maintenance treatment without maintaining sinus rhythm for more than two months. With the alprenolol treatment she remained in sinus rhythm for a little more than five months, relapsing to atrial fibrillation only a few days after withdrawal of this therapy Similar experiences have been made by Salokannel and Takkunen (20), who in 17 patients, all previously converted and relapsed in spite of maintenance quinidine therapy had 45% of the patients still in sinus rhythm at three months after conversion.

The fact that as many as 6 of the 11 patients maintaining sinus rhythm for more than three months did so on a daily alprenolol dose of 200 mg, or in some cases 300 mg, indicates that the dose is not critical in the patients doing well on this treatment. Of course, in order to get proof of the true role of the drug, one would have had to make a withdrawal experiment. How ever when it comes to explaining our poor a er age results, we cannot suggest any better explanation than that of unmasking a agal stimulation as cited above (9) perhaps even more so at our higher dose level.

Various arrhythmias have been described to occur in connection with a DC-con ersion. The risk is considered to be larger if maintenance digitalis treatment is not withdrawn a few days before DC-con ersion (3). Åberg and Cullhed (?8), as well as Szekely et al. (24) have analysed the types and frequencies of post-con ersional arrhythmias in detail. Compared to their experiences, our patients ha e had a lower incidence of extrasystoles (ventricular and supraventricular), nodal rhythm, ventricular tachycardia and atrial ventricular block, in spite of continued digitalis treatment. Similar experiences have been made by Szekely et al. (24). Linko et al. (13) have used alprenolol for the treatment of extrasystoles after DC-conversion in four cases with good results. That the low incidence of arrhythmias may be due to the treatment of an adrenergic β-blocker is in accordance with the results of Wittenberg and Lown (26) In dogs they showed that the myocardial irritability for an electric shock induced by digitalis was decreased by an adrenergic β-receptor blocker They consider this effect to be due both to β-blockade and, perhaps mostly to the previously mentioned direct membrane ef fect.

Warnings have been issued against the use of β-receptor blocker in connection with DC-conversion on the grounds that sinus node depression with sino-atrial block and sinus bradycardia might occur (15 24) These experiences are based on studies in which propranolol in doses (with regard to β-blockade) corresponding to roughly half of our alprenolol doses was used. We saw in one patient a short asystolia with spontaneous remission. In this case we could not rule out the possibility of a high digitalis maintenance dose being a contributory factor Apart from that, we noted neither dangerous nor a high incidence of bradyarrhythmias, nor ha e Hillestad and Andersen (9) reported any particular problems with post-conversional arrhythmias, using a daily dose of 160–200 mg of alprenolol. This adrenergic β-blocker has a slight β-stimulating effect (30) a property which propranolol does not ha e. This β-receptor stimulating effect could explain a difference between alprenolol and propranolol with regard to the incidence of post conversional sinus node inhibition. From the practical point of view this aspect may be of importance, e.g. fo angina pect ris patients on treatment with β-blockers, who could become candidates for DC-conversion. Our experiences indicate that it is not necessary to withdraw alprenolol treatment before the con ersion.

Finally one may ask whethe adrenergic β-receptor blockade has any place at all in the therapy to prevent relapse to chronic atrial fi-

brillation after DC-conversion. At least one of our cases illustrates that in selected cases it may be a valuable alternative when other drugs have failed. Some authors have reported (21 22, 23) very good results with the combination of quinidine or procainamide and an adrenergic β-receptor blocker when the dosage of each drug had been kept relatively low Hillestad and Storstein (10) have not been able to reproduce these results—on the contrary they noticed some serious complications with that type of therapy Our limited experience with the combination of quinidine and alprenolol does not indicate that this combination is of any greater value than the treatment with quinidine alone

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AVAILABILITY OF IRON STORES BUILT UP BY IRON DEXTRIN

As Studied with Desferrioxamine and Phlebotomy

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Abstract. Iron stores built up by iron dextrin have been studied in a group of iron-depleted, non-anemic blood donor volunteers. The chelatability by desferrioxamine (DF) of these iron stores was repeatedly examined during 21 months at different loads of administered iron. The availability for DF chelation was lower as compared to natural iron stores of comparable size. The availability for Hb formation was studied with vigorous phlebotomy and was found to be reduced. An iron-deficient state developed when approximately 65% of the administered iron was mobilized. Non-available iron deposits could be demonstrated in bone marrow smears in all subjects at the end of performed phlebotomies.

One of the main clinical indications for parenteral iron therapy is replenishment of iron stores in patients with chronic blood loss. The accretion of normal iron stores by oral iron therapy requires a long time and is usually considered difficult to achieve (10, 21, 41). Although it is well documented that parenteral iron preparations are effective in the treatment of iron deficiency anemia (1, 9, 28), some observations indicate that iron stores built up in this way are not completely available for Hb synthesis (5, 10, 15, 24). This problem has, however, not been sufficiently studied and no data exist as to the degree of availability of such iron stores.

The purpose of the present investigation was to study quantitatively the availability for Hb synthesis and the chelatability of iron stores built up by iron dextrin infusion. The availability for Hb synthesis was studied by the method of vigorous phlebotomy according to Haskins et al. (21), and the chelatability by means of the desferrioxamine (DF) urinary excretion test (17, 23, 27). The latter was studied at different iron loads and at different intervals after the iron dextrin infusion.

Iron dextrin (Ferrigen, Astrafer) is a colloidal solution of ferric hydroxide in complex with partially hydrolyzed dextrin. The complex has a mean molecular size of about 230 000. It is given intravenously in doses of 100 mg. After administration the material leaves the plasma in less than 24 hours (1). Its clinical effects have been studied by Andersson (1), Fielding (13) and Swedberg (38).

MATERIAL

Group I consisted of 33 male healthy volunteers, in whom a DF test was performed. They had a mean Hb concentration of 14.7 g/100 ml with a range of 13.3 to 16.5 g/100 ml. None had a history of hemorrhage or blood donation, and none had undergone gastric resection.

Group II consisted of 30 male blood donors, who for five years or more had donated regularly five to six units of blood per year. The DF test was performed about two months after the last donation. From this group ten blood donors were selected for the iron dextrin study (group II b).

Group II b. The mean number of blood donations for this group was 52 U (range 33–77). During the last two years they had donated at least 5 U/year and had not received iron supplementation. Their mean Hb concentration was 14.0 g/100 ml (range 12.4–15.6).

Group III consisted of 4 male volunteers, in whom iron deficiency anemia was induced by repeated weekly phlebotomies. Their iron stores were considered depleted when no rise in Hb concentration occurred two to three weeks after completion of the series of phlebotomies. Their mean serum iron was low (32 µg/100 ml) and the total iron binding capacity (TIBC) elevated (438 µg/100 ml) (Table I and Fig. 2).

METHODS

All blood specimens were taken in the morning by venous puncture with the subject in the recumbent position and in the fasting state.

Table I *Hb level, serum iron, TIBC and DF-induced urinary iron excretion in the groups studied (means and S.E.M.)*

Group	No. of subjects	Hb (g %)	Fe/s (µg %)	TIBC (µg %)	DF-induced iron excretion mg/24 h	DF-induced iron excretion µg/kg/24 h
I	33	14.7±0.1	131±7	331±7	0.78±0.03	10.3±0.3
II	30	14.5±0.2	101±5	374±10	0.45±0.02	6.2±0.2
IIb	10	14.0±0.3	110±10	388±19	0.44±0.03	5.9±0.4
III	24	10.0±0.1	32±2	438±10	0.36±0.01	4.9±0.1

The Hb was determined photometrically as cyanmet hemoglobin (12). Serum iron was determined according to the method of Laurell as described by Weinfeld (40), and TIBC according to the method of Peters et al. (32). The smears from the liver and sternal marrow were stained for hemosiderin according to the method of Hansen and Weinfeld (19).

Blood volume and total Hb mass were determined by isotope dilution technique using radioactive chromium (36).

The DF test was performed as follows: 500 mg of desferrioxamine B-methanesulfonate was dissolved in 5 ml of sterile water and administered in a dosage of 10 mg/kg b.wt. Approximately one half of the solution was given intragluteally on one side and the rest on the other side. To prevent pain and accumulation of the solution in a small muscular volume, the injection needle was withdrawn about 2 cm during the injection. The urine was then collected for 24 hours in iron-free polyethylene bottles. Ferrioxamine iron in the urine was determined in duplicate according to the method of Keberle (25) as modified by Lundvall and Weinfeld (27). The results are given in µg of urinary iron/kg b.wt. and in mg/24 h.

Iron dextrin was given in a slow i.v. injection of 100 mg. Blood was redrawn into the syringe two to three times to be certain that all iron in the syringe had been injected.

The DF-chelatability of iron stores built up by iron dextrin

Design of study. The study was performed on the 10 blood donors of group IIb. Before iron administration a DF test was performed. Then 550 mg of iron dextrin was injected over a period of 6 days and the second, early DF test was performed 3 days after the iron administration. A third, late DF test was done two months later.

In six subjects the study was continued. Two months after the first iron administration a second series of 5 × 100 mg of iron dextrin was given, and an early and late DF test were performed at the same time intervals as before. Five months after the first iron administration a third series of 10 × 100 mg of iron dextrin was given over a period of two weeks and again a DF test was performed early and late after the last injection. The [illegible]



Fig. 1 Mean values of Hb concentration, serum iron, TIBC and DF iron excretion in six blood donors during rebuilding of iron dextrin stores and during subsequent mobilization of the iron stores by phlebotomy

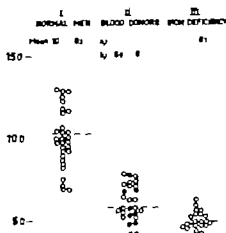


Fig. 2 DF-induced urinary iron excretion (µg/kg b.wt./24 h) in normal males, blood donors and in iron deficiency induced by phlebotomy. ● = values of blood donors who subsequently obtained iron dextrin.

Table II. *Relation of DF-induced urinary iron excretion to different loads of iron dextrin and at different intervals after iron administration (means and S.E.M.)*

N = 6. The figures in parentheses = values for the initial 10 subjects

Time (mo.)	Hb (g%)	Fe/s (μg %)	TIBC (μg %)	DF-induced iron excretion mg/24 h	DF-induced iron excretion μg/kg/24 h	Total amount of iron dextrin (mg)
0	14.0 ± 0.4	106 ± 14	393 ± 32	0.43 ± 0.03	6.5 ± 0.4	0
	(14.0 ± 0.3)	(110 ± 10)	(388 ± 19)	(0.44 ± 0.03)	(5.9 ± 0.4)	0
½		133 ± 10	370 ± 23	0.54 ± 0.05	7.1 ± 0.5	550
		(128 ± 10)	(364 ± 17)	(0.55 ± 0.04)	(7.4 ± 0.4)	550
2½	14.3 ± 0.3	151 ± 15	347 ± 15	0.56 ± 0.04	7.4 ± 0.5	550
	(14.1 ± 0.3)	(134 ± 12)	(347 ± 12)	(0.56 ± 0.02)	(7.6 ± 0.4)	550
2½		146 ± 21	325 ± 16	0.65 ± 0.06	8.7 ± 1.0	1 050
4½	14.9 ± 0.2	116 ± 11	368 ± 24	0.69 ± 0.05	9.1 ± 0.7	1 050
5		165 ± 12	337 ± 10	0.73 ± 0.06	9.6 ± 0.7	2 050
7	14.9 ± 0.2	145 ± 12	315 ± 15	0.79 ± 0.06	10.3 ± 0.7	2 050
13	15.0 ± 0.5	129 ± 13	309 ± 22	0.89 ± 0.07	11.7 ± 0.9	2 050
17[a]	13.9 ± 0.2	133 ± 10	299 ± 18	0.91 ± 0.04	12.0 ± 0.5	2 050
21	10.1 ± 0.2	40 ± 7	421 ± 23	0.35 ± 0.04	4.6 ± 0.5	

[a] At start of phlebotomy. [b] After phlebotomies.

group was then followed with repeated DF tests for 11 months. Subsequently the iron stores of the six subjects were mobilized by phlebotomies as described below and the DF test was repeated at regular intervals. The outline is given in Fig. 1 and Table II.

Quantitatr determination of the availability of iron stores built up by iron dextrin

Design of study. Six blood donors of group II *b* who were considered to possess negligible iron stores, received iron dextrin infusions as described above. Since they had no anemia, it was anticipated that virtually all of the iron dextrin given would be placed as storage iron. The availability of this storage iron for Hb synthesis was then determined by repeated phlebotomies according to the method of Heikins et al. (21). Phlebotomies of 445 ml were repeated at weekly intervals until the Hb concentration one week after the last phlebotomy had fallen to about 10.5 g/100 ml or below this level. If during the next two to three weeks the Hb level was unchanged, the serum iron persistently low and the DF test indicated iron deficiency it was concluded that the mobilizable iron stores were depleted. If the Hb concentration showed significant rise the phlebotomies were repeated until signs of iron depletion were achieved. About three weeks after the last phlebotomy sternal marrow aspiration was done. I two subjects fine needle aspiration liver biopsy was performed (39), and in one of them also an aspiration biopsy by the Menghini technique. The blood volume and the total Hb mass were determined three weeks after the last phlebotomy and were considered to be the same as at the start of phlebotomy (21).

Storage iron readily available for Hb synthesis was calculated as follows: Total Hb removed – Hb deficit – Hb synthesized. Hb deficit = initial total Hb – final total Hb. Hb synthesized (g) 3.4 – mg of iron used for Hb synthesis – absorbed iron (considered to be 3 mg/day) (15) = mobilizable storage iron.

After completion of the phlebotomies 75 mg elemental iron as ferrous succinate was given orally twice daily for two to three weeks. In five of the above volunteers second sternal marrow aspiration was performed ten months later. During the five to six months preceding the second bone marrow aspiration a new series of phlebotomies of 1 unit every month was performed.

RESULTS

Chelatability for desferrioxamine (Figs. 1 and 2, Tables I–III). The mean DF-iron excretion of blood donors (5.9 ± 0.4 μg/kg or 0.44 ± 0.03 mg) was significantly lower than that of normal men (10.3 ± 0.3 μg/kg or 0.78 ± 0.03) and significantly higher than that of the group with iron deficiency anemia (4.9 ± 0.1 μg/kg or 0.36 ± 0.01 mg).

The results of the group who received iron dextrin are given in Table II and Fig. 1. The DF test performed a few days after the administration of the first 550 mg of dextrin iron showed a small but statistically significant rise in the DF-iron excretion (early DF test). The DF-iron excretion was essentially unchanged two months later. After the second series of 500 mg iron dextrin infusion (total 1 050 mg) again a slight rise in the early DF test occurred. Two months later there was a small increase in the DF induced iron excretion.

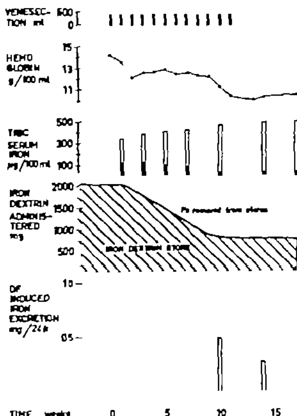


Fig. 3 Effect of weekly phlebotomies on Hb concentration, serum iron, TIBC, storage iron and DF-induced iron excretion in subject 1

After the third series of 1 000 mg iron dextrin injections (total 2 050 mg) the early DF test showed a negligible and insignificant increase in iron excretion in spite of an approximately 100% increase of the iron dextrin content in the body. At this point, with an iron dextrin load in the depots of 2 050 mg, the mean DF-induced iron excretion was 0.73 ± 0.05 *mg/24 h, which is lower* than the mean of normal men (0.78 ± 0.03). During the following months there was, however a successive rise, and 12 months after the last iron dextrin infusion the DF-induced iron excretion amounted to 0.91 ± 0.04 mg/24 h. The last value was, however still not significantly different from the mean of normal men (Table II, Fig. 1). A mean increase of Hb of 1 g/100 ml, which corresponds to a utilization of approximately 150 mg *of iron was noted during the period of iron* administration.

After phlebotomies the DF-induced iron excretion decreased to values found in iron deficiency anemia (0.35 ± 0.04)

Availability for hemoglobin synthesis (Fig. 3, Tables III and IV) Fig. 3 illustrates the changes in Hb concentration, serum iron and TIBC and *DF tests during phlebotomies in a typical subject.* During the first two weeks the phlebotomies induced a considerable decrease in Hb concentration. Then the Hb concentration levelled off at 12–12.5 g/100 ml, indicating that Hb production was equivalent to the amount of Hb removed by phlebotomy. This corresponds to an excess weekly delivery of iron from stores of 150–200 mg. After the ninth week, however further phlebotomies induced a sharp decrease in the Hb level, serum iron fell to values below 40 µg/100 ml with a transferrin saturation of below 8%. The DF induced iron excretion was within the range of iron deficiency anemia. At this point iron stores were considered not to be available for the erythroid marrow. Table II and Fig. 1 show the mean values for serum iron, TIBC and DF tests before and after phlebotomies. The amount of storage iron that could be mobilized in response to phlebotomy averaged 1 330 mg, with a range of 1 150 to 1 740 mg (Fig. 4). This corresponds to a *mean of 65% of the injected dose of 2 050 mg* iron dextrin. The increase in total iron binding capacity paralleled the decrease in mobilizable storage iron

During the state of iron-deficient erythropoiesis reticular iron could be demonstrated in the bone marrow smears of each *subject in* an amount similar to that seen in normal men. The iron deposits had a characteristic appearance of small, uniform, equal-sized granules in RE cells, sometimes aggregated into clumps (Fig. 5). Amorphous iron in RE cells could not be detected and the sideroblast count was almost zero. Cytological examination of iron-stained smears from fine needle aspiration biopsy specimens of the liver

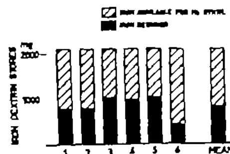


Fig. 4 Availability for Hb synthesis of iron stores built up by iron dextrin.



Fig. 5 Residual iron deposits in RE cells of the bone marrow aspirated in the state of phlebotomy-induced iron deficiency anemia in subject who had received 2 050 mg iron dextrin.

Table III. *Hb level, blood indices, serum iron, TIBC and DF-induced iron excretion at different intervals after administration of 2 050 mg iron dextrin*

Subject no.	Age (y.)	Weight (kg)	Stage of experiment	Time (mo.)	Hb (g %)	Hct (%)	MCHC (%)	Fe/s (µg %)	TIBC (µg %)	DF-induced iron excretion mg/24 h	DF-induced iron excretion µg/kg/24 h
1	32	70	Before iron admin.	0	15.2	48	32	167	506	0.36	5 1
			After iron admin.	6	15 4	48	32	118	365	0.77	11 0
			Before phlebotomy	16	14.0	46	30	128	348	0 80	11 4
			After phlebotomy	19	10 6	36	29	50	513	0.26	3 7
2	32	76	Before iron admin.	0	14 3	46	31	100	406	0.53	7 0
			After iron admin.	7	14.8	46	32	145	340	0.77	10.1
			Before phlebotomy	16½	14.5	47	31	137	301	1.02	13 4
			After phlebotomy	21	9 9	32	31	61	465	0.33	4 3
3	33	85	Before iron admin.	0	14 1	46	31	107	387	0.46	5 4
			After iron admin.	7	15.7	49	32	205	361	0 92	10.8
			Before phlebotomy	16½	14 2	46	31	136	295	0 92	10 8
			After phlebotomy	21	9 4	33	29	25	415	0 28	3 4
4	32	72	Before iron admin.	0	13 9	44	32	104	410	0.30	4 2
			After iron admin.	6	16 2	48	34	167	310	0 63	8.8
			Before phlebotomy	18	13.8	43	32	177	290	0 85	11.8
			After phlebotomy	21	10 0	34	29	36	360	0.30	4.2
5	33	77	Before iron admin.	0	13.2	43	31	74	367	0 53	6.9
			After iron admin.	7	15 0	46	33	174	326	0.76	9 9
			Before phlebotomy	19	14 0	44	32	104	351	1.05	13 6
			After phlebotomy	22½	9.8	32	31	19	386	0 44	5.7
6	32	75	Before iron admin.	0	13 4	45	30	140	265	0.37	5.1
			After iron admin.	5½	13.9	45	31	184	311	0 50	6.7
			Before phlebotomy	16½	12.9	43	30	113	248	0.82	10.9
			After phlebotomy	20	10 9	36	30	51	389	0 47	6 3

showed the same granular iron deposits. These granules were spread extracellularly all over the smear and their localization was impossible to determine. Sections from the Menghini biopsy specimen revealed that the granular iron deposits were localized entirely to the Kupffer cells. No iron was found in liver parenchymal cells.

In five subjects a second bone marrow aspiration biopsy was made ten months later. The latter was done after a new series of five phlebotomies performed at monthly intervals. In spite of an iron deficiency state, the granular iron deposits were still present in the reticulum cells. Their amount was, however smaller than in the former biopsy specimens, and were graded as trace to 1+

Table IV *Availability of iron dextrin stores for Hb formation in response to phlebotomy in six subjects*

Subject no.	Iron dextran administ. (mg)	Fe content of Hb removed by phlebotomy (mg)	Fe content of Hb deficit (mg)	Fe abs from food[a] (mg)	Fe mobilized from stores: Corr. for Fe abs. from food (mg)	Corr. for Fe abs. from food (% of Fe stored)	Not corr. for Fe abs. (mg)	Not corr. for Fe abs. (% of Fe stored)	Stainable iron in bone marrow smears after phlebotomy: Reticular (grade 0–4+)	Sideroblasts %
1	2 050	2 318	504	399	1 415	69	1 814	88	1+	0
2	2 050	2 630	841	420	1 369	67	1 789	87	2+	0
3	2 050	2 423	846	420	1 157	56	1 577	76	1+	0
4	2 050	2 102	576	342	1 184	57	1 526	74	2+	0
5	2 050	2 130	714	267	1 149	56	1 416	69	1+	0
6	2 050	2 434	396	297	1 743	85	2 040	99	2−	0

[a] Assumed to be 3 mg/day during the phlebotomy period (15)

DISCUSSION

The results have shown that the DF test could separate groups with iron stores of different size. This is in agreement with the results reported by Ploem et al. (33) and Hallberg and Hedenberg (17) The DF induced iron excretion was very low in blood donors and only slightly higher than in the group of iron deficiency anemia. The difference was, however statistically significant. This is in accordance with the results of chemical determinations of non-heme iron in bone marrow for these groups of subjects (40) Results from a preliminary study on mobilizable iron stores in 10 blood donors gave values ranging from 0 to 240 mg with a mean value of 100 mg (31) During the course of iron administration the mean Hb value increased 1 g/100 ml, corresponding to approximately 150 mg of iron This amount is probably of the same order as the size of the initial stores. It is therefore justified to assume that the total amount of dextrin iron administered to these blood donors was placed as storage iron.

The first two series of iron injections gave a small but significant rise in DF iron excretion. After the third series of 1 000 mg of iron dextrin the DF test performed immediately afterwards did not show any significant increase in iron excretion despite the fact that the iron stores increased by 100% This indicates that, when iron dextrin is administered to an individual with replenished iron stores, the added dextrin iron is not available for DF chelation.

The mean size of mobilizable iron stores in man is probably less than 1 000 mg. According to Balcerzak et al. (2) values above 1 500 mg are unusual Preliminary studies in six healthy adult men have shown a mean value of 665 mg with a range from 250 to 990 mg (31) Previous studies in this laboratory have shown that there is a linear regression of DF iron excretion on mobilizable iron (26) Similar results have been reported by Balcerzak et al. (2) According to these results a DF induced iron excretion of 2 mg should be expected in subjects with iron stores of 2 000 mg. The maximal mean DF iron excretion in the subjects loaded with 2 000 mg of dextrin iron was, however only 0 91 mg/24 h The value was still lower when the test was performed shortly after the iron infusion (0 73 mg/24 h) It is of interest to note that the chelatability increased successively with time. During the year following iron infusion a slow but significant increase in DF-iron excretion was noted (Table II and Fig. 1) indicating either a shift of iron to a compartment where chelation can take place or that the iron complex was physically or chemically transformed into a compound which is more accessible for DF chelation.

Mobilization of these iron stores by vigorous phlebotomy has shown that iron stores built up by the iron dextrin complex had a decreased availability for Hb synthesis. A state of iron-deficient erythropoiesis had developed when approximately 65% of the administered dose was mobilized. The serum iron was low TIBC elevated, and the DF test showed values as in iron deficiency During this state residual iron deposits were demonstrated in the bone marrow and RE cells of the liver In this respect this iron differs from natural iron stores which usually are completely available for Hb synthesis in response to phlebotomy (26 30 31) Even the large amounts of iron stored in hemochromatosis have been reported to be completely mobilized by phlebotomies (3 6, 11) However some cases of hemochromatosis have been reported in which residual iron deposits have been demonstrated after phlebotomy (6 11) This is probably due to the fact that some portions of natural storage iron might have physical properties which make them less available for Hb synthesis. According to *Weintraub* et al. even such deposits can be mobilized after some time (44) In the subjects of the present study residual iron deposits could be demonstrated a year later in spite of a prolonged period of erythropoietic stimulation by new series of phlebotomies. The amount of granular iron found on the final examination was, however smaller

Thus, iron stores built up by intravenous iron dextrin differ from natural storage iron in their decreased availability for desferrioxamine and transferrin. This might be due to a difference in the distribution and/or quality of iron dextrin stores as compared to natural ones. After injection, iron dextrin leaves the plasma in less than 24 hours and is taken up by RE cells. Then the complex has to be broken up before iron can be delivered to transferrin or move to other compartments. Few studies have been made on the further distribution of iron dextrin in man. Fielding (14) reported one case with bronchial carcinoma and iron deficiency anemia treated with

1 000 mg iron dextrin. At autopsy six weeks later most of the iron was found in the spleen and the liver. In the liver all iron was confined to Kupffer cells. No iron was found in liver parenchymal cells. This is in accordance with the distribution of saccharated iron oxide reported by Finch et al. (16). Animal experiments with colloidal iron preparations have shown that after deposition in Kupffer cells a redistribution of iron to liver parenchymal cells takes place. This transfer is slow and even after heavy iron overload most of the injected iron is confined to RE cells (7, 29, 34). The marked RE distribution of colloidal iron preparations is in contrast to the situation in normal males, in whom most of the stainable liver iron is found in parenchymal cells while stainable iron in Kupffer cells is usually scarce or absent (43). Studies by Harker et al. (20) suggest that iron in the liver parenchymal cells is the main source of DF chelation. A probable cause of the low DF chelatability might therefore be the RE distribution of the iron dextrin compound. The second possibility is that iron stores built up by colloidal iron preparations have other qualities and that they are rather slowly transformed to natural iron. The stainable iron deposits in bone marrow after colloidal iron administration have a characteristic appearance. They appear as small, uniform, equal-sized granules in the RE cells (5, 22, 42). One cannot assess whether these iron granules are true hemosiderin or merely represent the phagocytosed colloidal iron complex. Richter (35) has shown by electron microscopic studies on mice that, during the first 6 days after parenteral iron injections, iron is deposited in the tissues mainly as colloidal iron. About 3–4 weeks later ferritin and hemosiderin predominate and only traces of colloidal iron residues are present. It is not known whether the events in man follow the same pattern.

The end result of phlebotomies was a state of iron deficiency anemia with residual iron deposits in the bone marrow. This picture has been described previously in subjects treated with saccharated iron oxide (5, 10, 15, 22, 37). Henderson and Hillman (24) found that iron stores built up by iron dextran showed in time a decreased availability for hemoglobin production, which is in agreement with our unpublished results (31).

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ON THE METABOLISM OF FAT AND CARBOHYDRATE IN A PATIENT WITH REFRACTORY SIDEROBLASTIC ANAEMIA

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Abstract. A patient with an acquired refractory sideroblastic anaemia has been studied. The anaemia had many features in common with pernicious anaemia, but high levels of vitamin B_{12} and normal urinary excretion of methylmalonate excluded vitamin B_{12} deficiency. A decreased pyruvate utilization and an increased urinary excretion of acetate were demonstrated. Studies in vitro with white blood cells indicated decreased efficiency of tricarboxylic acid cycle oxidation due to low citrate synthetase activity. All serum lipid fractions were low and decreased lipolytic response to exercise and isoprenaline infusion was observed. The pathogenesis of these changes is discussed.

The anaemia in vitamin B_{12} deficiency is probably due to the absence of a not fully understood effect of the vitamin on the synthesis of DNA (7). Vitamin B_{12} also plays a role in the isomerization of methylmalonyl-CoA, a reaction which requires the deoxyadenosine derivative of the vitamin as cofactor (8, 13). Pernicious anaemia is characterized by an increased urinary excretion of methylmalonate (1, 4) and its precursor propionate (5).

Deficiency of vitamin B_{12} is also associated with high fasting levels of pyruvate and low levels of 2-oxyglutarate. Following the administration of glucose the rise in blood pyruvate is abnormally high, whereas the normal rise in 2-oxoglutarate is absent (3). Cox et al. (5) observed an elevated excretion of acetate in the urine of patients with pernicious anaemia and concluded that deficiency of vitamin B_{12} may be associated with a decreased oxidative efficiency of the citric acid cycle.

In a recent report (12) the haematological and cytogenetical findings were described in a patient with refractory sideroblastic anaemia. Despite high levels of vitamin B_{12} in serum the patient's anaemia showed several features that are characteristic of a deficiency of this vitamin. In view of these similarities it was considered of interest to investigate whether the patient showed any of the other metabolic abnormalities that may occur in pernicious anaemia.

CASE REPORT

The patient, 57-year-old fitter, has been described in detail in previous paper (12). He as essentially healthy until 1967 when he began to complain of easy fatigue, palpitations, exertional dyspnoea and headache. On admission in 1967 he looked pale but his general condition was good. There was no atrophy of the tongue, no adenopathy nor were the liver or spleen palpably enlarged. Neurological examination, including vibratory sensation, was normal. There was no malabsorption or malnutrition (height 168 cm, eight 69 kg).

On admission Hb was 6.4 g/100 ml, Hct 22%, red blood cells 2.2 $10^6/mm^3$ and reticulocyte count 0.7%. The white blood cell and the thrombocyte counts were normal. The MCV was elevated and the MCHC was low normal. The serum iron was 318 μg/100 ml and the transferrin saturated. Haptoglobin in serum was 36 mg/100 ml but the LDH was normal. The concentration of vitamin B_{12} in serum was repeatedly elevated and Schilling test showed 23% urinary excretion of orally administered Co^{58} cyanocobalamin within the 24 hours. The excretion of methylmalonate in the urine was normal. Folic acid in blood was low normal.

Examination of the bone marrow revealed marked erythroid hyperplasia, whereas the myelopoiesis and thrombopoiesis appeared normal. The erythroid cells were large and often binucleated. Mitotic figures were common. Polychromatic cells were numerous. Some of them were of the ordinary normoblastic type but the majority had megaloblastic features. In the electron microscope the mitochondria of the erythroblasts appeared swollen. The cristae were ruptured or absent and contained clumps of opaque material, probably iron. Of the Hb in peripheral blood 15% was made up of HbF.

Table I *Laboratory data on carbohydrate and fat metabolism*

	1967	1968	1969	Normal range
Blood glucose (mg/100 ml)	69	80	71	60–95
Blood lactate (μmoles/ml)	0.86	1.32, 1.98	1 77	0 4–1 0
Blood pyruvate (μmoles/ml)	0.13	0.10 0 14	0 15	0 03–0.10
Lactate/pyruvate ratio	6.8	13.2, 14 1	12.0	5–15
Serum cholesterol (mg/100 ml)	130 (116–144)	120 (104–135)	76 (55–100)	140–280
Serum triglyceride (mg/100 ml)	80	65, 69	56, 80	60–180
Serum phospholipid (mg/100 ml)	—	146	—	150–300
Plasma free fatty acids (nmoles/ml)	—	154 303 407	474, 263	250–750
Blood 3-hydroxybutyrate (μmoles/ml)	0.017	0.031 0 060		0.005–0 10
Blood acetoacetate (μmoles/ml)	0 075	0 062, 0 083		0.005–0 06
3-hydroxybutyrate/acetoacetate ratio	0 2	0.5, 0.7		0.5–2.5

A large population of cells with 47 chromosomes and an extra C chromosome was found in the bone marrow cells but not in skin cells or lymphocytes The concentration of uracil nucleotides in the bone marrow of the patient was increased. There was low incorporation of thymidine into erythroblasts, which has also been observed in pernicious anaemia (12, 19 20). A considerable number of early erythroblasts not incorporating thymidine had an intermediate DNA content indicating an arrest in DNA synthesis. A normal labelling index was recorded for the myelopoietic cells.

Various therapeutic trials (pyridoxine, yeast, pig thymus, folic acid, tocoferol, prednisolone biotin, methylmalonate, lipoic acid and vitamin Q) were performed for periods of 3 weeks. No effect on reticulocyte count, Hb concentration or serum iron was ever observed.

METHODS

Routine analyses were performed at the clinical laboratory at Serafimerlasarettet. Gas chromatography was used for the determination of plasma free fatty acids (9), organic acids in blood and urine (10) and *short-chain fatty acids* in serum and urine (15). Lipolysis in adipose tissue was studied in vitro according to Rosenqvist et al. (18).

Metabolic studies f blood cells in vitro The oxidative metabolism of circulating blood cells was studied by incubating whole blood or suspension of white blood cells together with labelled substrates. White blood cells were isolated after dextran sedimentation of erythrocytes

Table II. *Lipid content of erythrocytes (mg/100 ml packed erythrocytes)*

	Cholesterol	Phospholipid	Phospholipid/cholesterol ratio
Patient	184	371	2.0
Controls	202	381	1.9
	217	419	1.9
	179	406	2.3

and suspended in 0.9% saline at a final concentration of 10 000 to 15 000 cells/μl.

The incubations were carried out in 10 ml Erlenmeyer flasks provided with a central well. The outer compartment contained 1 ml Krebs-Ringer phosphate buffer (pH 7.2) and 0.5 ml blood or 0.5 ml of suspension of white blood cells in saline. The inner well contained 0 2 ml ethanolamine. Approximately 1 μCi of the labelled substrate was added to the outer wall of each flask. The incubations were carried out in duplicate during 60 min at 37°C under slow agitation. The per cent perchloric acid (0.5 ml) was then added to the outer well of the flasks, which were placed in an ice-bath for 2 hours. The content of the central well with the trapped $^{14}CO_2$ was then transferred into a liquid scintillation vial containing 4 ml ethylene glycol monomethyl ether and 15 ml scintillation fluid.

When studying lipid synthesis, the incubation was stopped by the addition of 2 ml methanol and the content of the vessel was extracted with 20 ml chloroform-methanol (2 1 /). Triglycerides were separated by silicic acid column chromatography

Determination of citrate synthetase activity in the blood cells. Suspensions of white blood cells were diluted with an equal volume of tris HCl buffer (pH 9.0) and the cells were homogenized in Model W185D Sonifier (Branson Sonic Power Co., Plainview N.Y.) for 90 sec During this procedure the cell suspension was cooled in an ice-bath. The solution obtained was clear and could be used directly for the assay Citrate synthetase activity was determined at 340 nm according to Ochoa (16). The cuvette contained 50 μmoles tris HCl (pH 8.0), 8 μmoles L-malate 0.4 μmoles NAD+ 1 μg antimycin A and homogenate corresponding to about 10^6 white blood cells. The reaction was started by adding 0.2 μmoles acetyl-CoA.

Labelled materials. Palmitic acid-1 ^{14}C (50 mCi/mM), sodium acetate-1 ^{14}C (40.0 mCi/mM), DL-citric acid-1,5-^{14}C (15 4 mCi/mM) and sodium 2-oxoglutarate-5-^{14}C (17 1 mCi/mM) were obtained from The Radiochemical Centre, Amersham, England Sodium pyruvate-2 ^{14}C (1.1 mCi/mM) was obtained from NEN Chemicals, Dreieichenhain, Germany The labelled substances were used without further purification.

RESULTS

Fasting blood glucose as well as oral and intravenous glucose tolerance tests were repeatedly within the normal range. Glucosuria was never observed. Fasting blood lactate and pyruvate were elevated (Table I). During an intravenous glucose tolerance test (25 g of glucose in a rapid injection) the blood lactate remained essentially unchanged, while the pyruvate level (Fig. 1) increased more than normally (11).

All serum lipid fractions were consistently below or in the low normal range (Table I). Lipoprotein electrophoresis showed a normal distribution between lipoproteins. The fatty acid pattern of the plasma free fatty acids (FFA) was normal. In contrast to the findings in serum, erythrocyte cholesterol and phospholipid content were in the same range as that observed in three control subjects (Table II). The urinary excretion of methylmalonate was measured on several occasions and never exceeded 1.5 mg/day. Fasting blood acetate was normal, 0.06 μmol/ml (15), while the excretion of acetate was greatly elevated, 1 900 μmoles/day (5). The excretion of propionate was within normal limits (9 μmoles/day).

The low plasma FFA responded subnormally to lipolytic stimuli. Exercising on a bicycle ergometer with low work intensity (200 kpm/min) exhausted the patient after 5.5 min and the exercise had to be stopped. During this period the arterial plasma FFA changed from 237 μmoles/l at rest to 242 μmoles/l at the end of the exercise; 5 min later it was 251 μmoles/l. Respiratory RQ increased from 0.76 during rest to 0.91 during exercise. Thus the expected rise in FFA due to increased lipolysis during and after exercise was not observed.

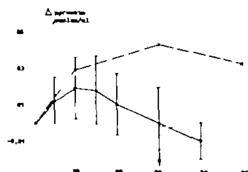


Fig. 1. Changes in blood pyruvate after intravenous administration of 25 g of glucose. the present patient, — means (±S.D.) in group of 1 control subjects with normal glucose tolerance (7).

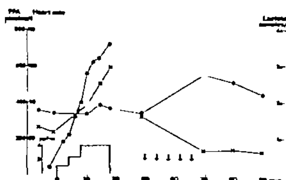


Fig. 2. Changes in heart rate (○—○), FFA (—), and blood lactate (●—●) induced by isoprenaline infusion (hatched area) and nicotinic acid administration (each arrow representing 0.1 g intravenously and 0.2 g orally).

The response of plasma FFA to isoprenaline was tested by giving a continuous intravenous infusion. The results are illustrated in Fig. 2. The infusion had a marked effect on heart rate but did not increase arterial plasma FFA above the normal fasting rate. Thirty minutes after the end of the infusion the plasma FFA had returned almost to the initial level. Upon administration of nicotinic acid (0.5 g intravenously and 1 g orally divided into 5 doses during 20 min) there was a further decrease of FFA to 120 μmoles/l (Fig. 2). After administration of this drug the patient ex

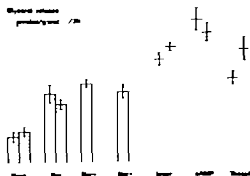


Fig. 3. Glycerol release from adipose tissue incubated in vitro. Open bars = the present patient, hatched bars = control subjects (= 6). Values are means ± S.E. Additions: Nor = 1-noradrenaline (5 μg/ml), PA = phentolamine (0.5 and 50 μg/ml), Isopr = 1-isopropylnor-adrenaline (5 μg/ml); cAMP = dibutyryl -AMP (10^{-4} M); Theoph = theophylline (10^{-4} M).

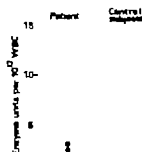


Fig. 4. Citrate synthetase activity in homogenates of circulating white blood cells. One unit is defined as the conversion of 1 mole of substrate/min at 25°C.

perienced an extreme muscular weakness for more than 24 hours.

Lipolysis in subcutaneous adipose tissue was studied in vitro (Fig. 3). The specimen from the patient responded normally to the lipolytic agents used.

The oxidative metabolism of the patient's white blood cells was studied in vitro. With labelled substrates that have to enter the citric acid cycle (palmitate, pyruvate and acetate) the production of $^{14}CO_2$ was consistently lower with cells from the patient than with cells from control subjects (Table III). Incubations were carried out in 1 ml Krebs-Ringer phosphate buffer (pH 7.4) to which were added 0.5 ml whole blood (B) or white blood cell suspension (WBC) and approximately 1 μCi of the labelled substrate. The addition of L-aspartic acid (1 and 10 μmoles/ml incubation medium) increased the production of $^{14}CO_2$ from acetate 1-^{14}C to the same extent as for controls. The addition of DL-lipoic acid (40 and 200 μg/ml incubation medium) had no significant effect on the $^{14}CO_2$ production of the patient's or the control cells. With labelled substrates that are intermediates in the citric acid cycle (citrate and 2-oxoglutarate) the tendency was reversed, the cells from the patient producing rather more $^{14}CO_2$ than control cells. Similar observations were made with cells from a patient with untreated pernicious anaemia. These findings are compatible with a defect in the citrate synthesis. A low activity of citrate synthetase in circulating white blood cells from the patient (Fig. 4) further supports the localization of the oxidative defect to this reaction.

The synthesis of labelled triglycerides from acetate 1-^{14}C was studied in one experiment. The recovery of added radioactivity in the triglyceride of 10^8 white blood cells of the patient, a patient with pernicious anaemia and two control subjects was 0.021, 0.013, 0.038 and 0.035% respectively. The figures given are means of two incubations.

DISCUSSION

The patient described in this and a previous paper (12) had an anaemia with many features in common with pernicious anaemia. High serum levels of vitamin B_{12} and the absence of an increased urinary excretion of methylmalonate excluded deficiency of this vitamin as a cause of his anaemia.

In similarity with previous findings in patients with pernicious anaemia there was an increased response of blood pyruvate after glucose administration. Another similarity was high excretion

Table III. *Recovery of $^{14}CO_2$ in per cent of added radioactivity per 10^8 white blood cells. The figures are means of two incubations each*

^{14}C-substrate	Cells	Patient	Untreated pernicious anaemia	Controls	
Sodium acetate-1-^{14}C	B	0.86		1.35	1.59
Sodium acetate-1-^{14}C	B	0.81		1.03	1.10
Sodium acetate-1-^{14}C	B	0.56		1.56	
Sodium acetate-1-^{14}C	WBC	0.22	0.22	0.80	0.54
Sodium acetate-1-^{14}C	WBC		0.37	0.60	0.91
Sodium pyruvate-2-^{14}C	B	0.065		0.10	
Palmitic acid-1-^{14}C	B	0.035		0.048	0.056
Citric acid-1,5-^{14}C	B	0.010		0.006	0.008
Citric acid-1,5-^{14}C	WBC		0.013	0.004	0.008
Sodium 2-oxoglutarate-5-^{14}C	B	0.015		0.013	0.011

of acetate in the urine. His white blood cells showed a low citrate synthetase activity. Upon incubation with such cells from the patient as well as from a patient with pernicious anaemia there was evidence of a decreased oxidative activity of the citric acid cycle. The results further indicated that this defect was probably located to the citrate synthesis reaction since the oxidation of substances that have to enter the citric acid cycle was depressed. The low synthesis of triglycerides from acetate in white blood cells from the patient as well as from the subject with pernicious anaemia is also consistent with a defective citrate synthesis, since this reaction appears to be involved in the transfer of acetyl-CoA across the mitochondrial membrane supplying substrate for the extramitochondrial fatty acid synthesis (14).

The serum lipid fractions were low in the patient, which was possibly a non-specific phenomenon since decreased levels of cholesterol and triglycerides have been reported in other types of anaemia (17). There was a low plasma FFA response both to isoprenaline infusion and during exercise. This may be due to some mechanism not operating in vitro, as evidenced by the incubation studies with adipose tissue. Ribonucleic acid and several nucleotides have been shown to inhibit lipolysis and to increase fatty acid reesterification in adipose tissue (6, 7). There is an interesting possibility that the patient's deranged nucleic acid metabolism (12) might influence his FFA levels.

In summary, the present patient, in spite of an abnormally high level of vitamin B_{12} in blood, showed many features characteristic of pernicious anaemia. The absence of an increased urinary excretion of methylmalonate and propionate in the patient demonstrates that the deoxyadenosine derivative of vitamin B_{12} functions normally as cofactor in the isomerization of methylmalonate. On the other hand there is evidence that the patient as well as subjects with pernicious anaemia have a defect in the peripheral metabolism, probably localized to the citric acid cycle. Vitamin B_{12} is not known to participate in any of the reactions of this cycle. These metabolic findings may therefore be unrelated to the availability of vitamin B_{12}. However, there is a possibility that some derivative of the vitamin is essential for the normal function of the citric acid cycle and that the patient is unable to synthesize that derivative.

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THE DETERIORATIVE EFFECT OF MYOCARDIAL INFARCTION UPON PHYSIOLOGICAL INDICES OF WORK CAPACITY

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Abstract. The present study has been performed in order to investigate the deteriorative effect of myocardial infarction upon physiological indices of work capacity. Sixteen male patients, averaging 52 years of age, have been tested 12 weeks after the acute episode by means of bicycle ergometry in the upright position. Each patient exercised at several loads, starting at low levels and increasing the loading until the criteria of maximality were reached. All patients tolerated well the strain of heavy muscular exercise. Their maximal oxygen uptake averaged 2.05 l/min or 26.8 ml/min/kg b.wt., which is about 25% lower than average for healthy men of corresponding age. The highest recorded heart rate averaged 163 beats/min, which is lower than for healthy people. A low maximal oxygen pulse indicates reduced stroke volume. These findings suggest a reduction of the pumping capacity of the heart. The arterial systolic BP showed a tendency to levelling-off at high levels of work rate, which suggests a reduced contractile power of the cardiac muscle. In 13 patients ECG changes indicating impaired oxygenation of the myocardium occurred when the work level exceeded two-thirds of their maximal aerobic power. At lower levels of submaximal exercise the physiological response pattern of the cardiacs was similar to that of normals, except for a tendency to higher pulmonary ventilation. The cause of this hyperventilation, which was related to an increased tidal volume, is suggested to be a change of the ventilation/perfusion rate brought about by the heart infarction.

Few investigations of the deteriorative effect of ischemic coronary diseases upon physiological indices of physical performance capacity have been carried out. Exercise testing is an accepted method in this evaluation, standard procedures have been worked out, and are recommended for use in clinical practice (21). These procedures are based on measurements taken during submaximal work, which is the only safe way of applying exercise tests in routine clinical work. However in order to evaluate physiological mechanisms involved in pathogenesis of impaired physical performance capacity associated with cardiac infarction, maximal exercise loading is required. Maximal exercise tests have been applied to cardiac patients by Kasser and Bruce (13) and by Kasch and Boyer (12).

In a previous publication (3) it was shown that when coronary patients are tested 20 months after myocardial infarction their maximal oxygen uptake is about 30% lower than the average of healthy people. The purpose of the present investigation was to broaden the knowledge of this deteriorative effect upon physiological indices at maximal exercise. For this purpose 16 patients who had recently suffered from a myocardial infarction were tested approximately 12 weeks after the acute cardiac episode.

MATERIAL

Seventeen men under 60 years of age suffering from their first myocardial infarction were randomly drawn from Medical Department IX, Ullevål Hospital, Oslo, for evaluation of their physical performance capacity. One of the patients was excluded from the material as he was unable to accommodate himself to the testing procedure and had difficulties in breathing through the respiratory valve. None of the remaining 16 patients were in particularly good physical condition before the infarction, and only one had an athletic background. They were all at work until admission to hospital. Nine of them were engaged on manual and seven on non-manual work. Some physical characteristics of the subjects are given in Table I.

Of the 16 patients six had an anterior infarction, two an anterolateral, seven a posterior and one a postero-lateral infarction. The severity and dimension of the infarctions varied. All but one patient had fever reaction during the first days in hospital. In the acute stage of the disease a pericardial friction rub was heard in four

Table I. *Physical characteristics of 16 male myocardial infarction patients*

	Mean	S.D	S.E.	Range
Age (y)	52	4.2	1.0	44–58
Height (cm)	174	5.7	1 4	164–182
Weight (kg)	76.4	8 4	2.1	60.5–92.0
Heart vol. (ml)	875	1 7	32	660–1 050
Heart vol. (ml/m²)	463	57	14	350–550

patients. Transient disturbances of the cardiac rhythm, sinus tachycardia in one, and premature supraventricular and ventricular beats in another of these four patients were observed. In two other patients multiple premature beats were recorded. On discharge from the hospital all patients had a normal cardiac sinus rhythm and none showed signs or symptoms of heart failure.

Most of the heart infarction patients were admitted to the hospital during the first hours after the heart attack, but some entered hospital on the first to fourth day after the onset of the acute illness. Therefore, the highest transaminase values given in Table II cannot be considered as peak values.

Four patients had diastolic arterial B.P higher than 100 mmHg when measured repeatedly at rest in the sitting position on the bicycle. In two of them a systolic pressure above 150 mmHg was recorded.

Only three subjects had cholesterol values higher than 300 mg/100 ml (Table II). None of them had xanthomatosis. Three patients had history of typical angina pectoris before the infarction episode In the other subjects the disease history revealed no symptoms of coronary heart disease prior to the acute episode.

According to the routine in Medical Department IX all the patients were treated with anticoagulants. The treatment was continued for six months to one year after discharge from the hospital. One patient was digitalized because of moderate and transient signs and symptoms of pulmonary congestion.

METHODS

Twelve weeks after the onset of myocardial infarction the testing of the patients' physical performance capacity was undertaken. A clinical examination including ECG with 12 leads in the supine position preceded the testing procedure. In all but one patient there were various ECG

Table II. *Some biochemical values in 16 male myocardial infarction patients*

Chemical test	Mean	S D	S.E.	Range
S-GOT (U)	231	114	28.5	51–414
Cholesterol (mg 100 ml)	266	44	11 4	182 357
Lactic acid after exercise (mg/100 ml)	69.2	14 4	3.6	45–97

changes compatible with a previous myocardial infarction. X-ray of the heart and chest in the erect position was taken at discharge from hospital, 3–4 weeks after the acute episode. The heart volume was determined according to Jonsell (11).

Maximal oxygen uptake and related respiratory and circulatory functions were measured by having the subjects bicycle on an ergometer of the mechanical braking type. On the first day of testing only one, occasionally two, moderate work loads were performed to accustom the patients to the work situation. On the following days two loads without intermittent rest period, either two submaximal loads, each lasting 5–6 min, or one submaximal and one maximal load, the latter lasting approximately 3 min, were performed daily for 3–5 days, until the criteria of maximality had been attained. The physiological measurements were taken in the last minutes of the exercise periods. If two maximal performances resulted in different values, the highest value was accepted.

Respiratory measurements were taken by using an open circuit system, expired air was collected into a balanced 150 l tank, and samples of gas withdrawn for analysis by means of the / ml Scholander method (19). Ventilation volume and respiratory rate were simultaneously recorded on kymograph.

ECG at rest was recorded with the subjects in the sitting position on the bicycle. Four CH leads, corresponding to V V V V_6 were used. During the exercise period the same four CH leads were regularly recorded at short intervals and compared to the resting ECG. Immediately after the exercise, and for 1 5 min afterwards, the procedure was repeated. Following maximal work load ECG was recorded at intervals for 10 min. Especially at maximal work loads an irregular base line and other disturbances could make the interpretation of the ECG difficult during exercise. A change from the pathological resting ECG ith either ST depression of at least 1 mm ith a horizontal or downward sloping segment, or a ST elevation of at least 2 mm, was considered abnormal. The heart rate was taken from ECG in the last minute of the working period.

Arterial B.P was measured in the upright sitting position on the bicycle before and during exercise by the cuff method and with microphone placed over the brachial artery The sound signal as transferred and amplified by means of an electronic device constructed by the author. The mean arterial B.P was calculated by adding one-third of the pulse pressure t the diastolic pressure.

Blood lactate was measured by the method of Scholander and Bradstreet (20) in venous blood sample hich was drawn 3–4 min after maximal performance.

None f the patients reported symptoms of angina pectoris during bicycling, and the aim as to load the patients in exercise so that levelling-off of oxygen uptake could be established. This criterion of maximality was convincingly demonstrated in 10 of the 16 patients. In the remaining 6 patients post-exercise blood lactate exceeded 59 mg/100 ml, and respiratory gas exchange ratio was higher than 1.06. In all cases the testing session was completed and ECG changes occurring during exercise were not considered to be an indication for interrupting the testing procedure.

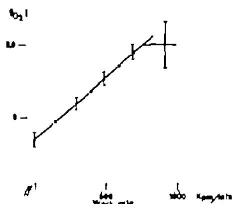


Fig. 1. Oxygen uptake/work output relationship in 16 ma myocardial infarction patients (mean values). Vertical lines denote ± S D

RESULTS

Oxygen uptak at submaximal work

The oxygen cost of bicycling at submaximal work rates is presented in Fig. 1 and Table III The mean values, as well as the interindividual variations, are similar to those in healthy men of corresponding age (2) and equal to values reported earlier for coronary heart patients (3)

Maximal oxygen uptake

The highest measured values for oxygen uptake during work are presented in Table IV and Fig. 2. The maximal O_2-uptake averaged 2.05 l/min, corresponding to 26.8 ml/min/kg body weight or 11.8 ml/min/cm body height. This is about 25% lower than average for healthy men of the same age. The coefficient of variation is about 15%

Lactic acid

The blood lactate measured 3–4 min after maximal performance averaged 69.2 mg/100 ml (Table II).

Heart rate

The resting heart rates averaged 68/min in the recumbent position, which increased to 77/min while seated on the bicycle.

Heart rate was found to be linearly related to oxygen uptake (Table V) in accordance with numerous published reports. The slope of the regression line tended to be steeper than in healthy men of corresponding age (Fig. 3) but at low work level the heart rate was not much different.

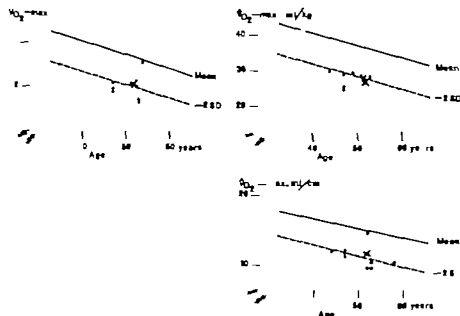


Fig. 2. Maximal oxygen uptake in relation to age given in absolute and relative values, related to body weight and body height. mean value for the cardiacs. The regression line for normal subjects is draw according to findings reported from the same laboratory (2, 9).

Table III. *Oxygen uptake during bicycling at submaximal work intensities*

Work rate		$\dot{V}_{O_2}$ (ml STPD) Mean	S.D	S.E.
N load	16	700	96	24
300 kpm/min	16	1 200	82	21
500 kpm/min	16	1 539	88	22
700 kpm/min	11	1 903	100	30

Values for highest recorded heart rate during exhaustive exercise, maximal heart rate are listed in Table IV and averaged 163 beats/min, with a range from 148 to 180. This is somewhat lower than found for healthy men (Fig. 4)

Maximal oxygen pulse

The highest values for oxygen pulse are given in Table IV and averaged 12.7 ml, which is somewhat lower than reported for healthy men of similar age (Fig 5)

Arterial blood pressure

At rest in the upright position, sitting on the bicycle, the arterial B.P averaged 129/92 mmHg, the average mean arterial pressure being 104 mmHg. The diastolic pressure is somewhat higher than in normals in the same position. Systolic and mean B.P increased in all subjects during work in close linear relationship to the rate of work.

Table IV *Highest recorded physiological variables during maximal muscular exercise*

$\dot{V}_{O_2}$-max = maximum oxygen uptake, HR = heart rate, $\dot{V}_E$-max = highest pulmonary ventilation during exhaustive exercise, V_t = tidal volume, f_{resp} = respiratory rate (breathing frequency), R = respiratory exchange ratio (RQ)

Variables	Mean	S.D	S.E.
$\dot{V}_{O_2}$-max (l)	2.05	0.34	0.09
$\dot{V}_{O_2}$-max (ml/kg)	26.8	3.45	0.86
$\dot{V}_{O_2}$-max (ml/cm)	11.8	1.78	0.45
HR-max (beats/min)	163	11.3	3.1
O_2-pulse-max (ml)	12.7	2.0	0.5
B.P systolic	183	19.7	4.9
B.P mean	124	14.4	3.9
B.P diastolic	94	13.6	3.7
$\dot{V}_E$-max (l)	66.3	9.6	2.4
V_t (l)	2.48	0.11	0.03
f_{resp}/min	26	3.7	0.9
R-max	1.11	0.07	0.02

Table V *Heart rate at various levels of oxygen uptake and work rate*

Load		Heart rate Mean	S.D	S.E.
$\dot{V}_{O_2}$	750 ml/ml	91	7.3	1.8
	1 000 ml/min	105	7.8	1.9
	1 250 ml/min	119	9.1	2.3
	1 500 ml/min	133	11.2	2.8
$\dot{W}$	No load	92	8.1	2.3
	300 kpm/min	115	8.9	2.2
	500 kpm/min	134	10.4	2.6
	700 kpm/min	148	12.0	3.6

When approaching maximal work levels a tendency to levelling-off in pressure response was noted (Fig. 6) The highest values for systolic arterial B.P averaged 182 mmHg, and the average mean pressure was 122 mmHg. In four patients systolic arterial pressure of 200 mmHg or higher was recorded in exhaustive exercise. The diastolic arterial pressure did not change much.

Heart volume

The volume of the heart averaged 875 ml or 463 ml/m² BSA (Table I) There are, however large interindividual variations in this parameter Four of the patients had values above 500 ml/m² and in one of them a value of 550 ml/m² was measured. The latter value is considered pathologically enlarged, according to Amundsen (1)

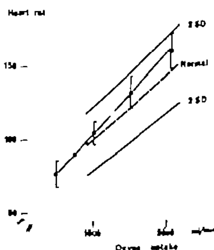


Fig 3 Heart rate/oxygen uptake relationship in myocardial infarction patients compared with normal men of the same age (2). Vertical lines denote ± SD for the cardiacs.

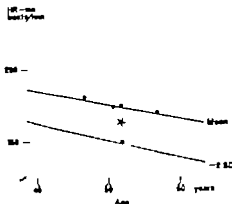


Fig. 4. Maximal heart rate in relation to age. ★ mean value for the myocardial infarction patients. The regression lines present normal values from the same sources as in Fig. 2.

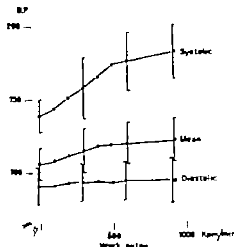


Fig. 6. Arterial B.P. response to increasing work rates in myocardial infarction patients (mean values). Vertical lines denote ±S.D.

The relationship between the heart volume and the maximal oxygen uptake and the maximal oxygen pulse is demonstrated in Fig. 7. The correlation coefficients are 0.51 and 0.41 respectively: this is to be considered a poor relationship. Estimating normality from this relationship, it is clear that a large fraction of the patients fall below the lower limit for normal sedentary men of the same age.

Electrocardiogram

In all but one patient the resting ECG showed, in a varying degree, changes from normal pattern. These changes were indicative of myocardial infarction. During and/or after maximal exercise tests, changes from the resting ECG occurred in 13 of 16 patients. The nature of these changes is presented in Table VI.

ST elevations were only recorded in patients with an earlier anterior myocardial infarction. No abnormalities in rhythm or conduction were noted, except for two intermittent and transient intraventricular conduction disorders during exercise. Single supraventricular and ventricular ectopic beats observed either at rest or during and after work load in a few patients were occasionally observed.

The relative load at which ECG changes occurred varied among the patients from 50% to 90% of the maximal oxygen uptake level, averaging 67% or in other words at a work level corresponding to two-thirds of their maximal aerobic power.

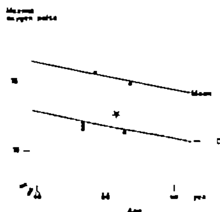


Fig. 5. Maximal oxygen pulse related to age. ★ mean value. The lines present the regression line for normals (2, 9).

Respiratory variables

The highest pulmonary ventilation during exercise averaged 66 l/min (Table IV) as compared to approximately 80 l/min in normals of the same age. Maximal voluntary ventilation (MVV) was assessed in only seven patients (data not reported) and in these patients the ventilation in maximal exercise required 63% of the MVV. Pulmonary ventilation increased in linear relationship to the increasing oxygen uptake up to 75–80% of the maximum, which is in agreement with observa

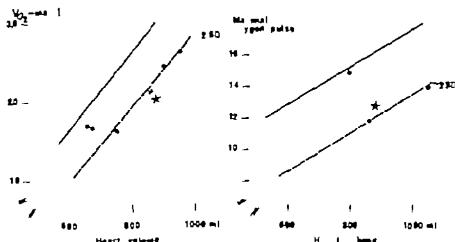


Fig 7 Relationship between heart volume and maximal oxygen uptake and maximal oxygen pulse in myocardial infarction patients. ★ mean values. The normal regression lines are drawn according to other investigators (14 15) with some corrections.

tions on healthy individuals (Fig. 8) The amount of hyperventilation was relatively similar to what is common in healthy subjects. However the pulmonary ventilation efficiency measured as ventilation equivalent, was poorer than in normals, and with regard to this characteristic the patients resembled old people (Fig. 9) (5) In Fig. 8 it is clearly demonstrated that the ventilation rate was higher than observed in healthy subjects.

The tidal volume (V) increased with the work rate (Fig. 10) The relationship to the oxygen uptake was rather poor ($r = 0.60$) The average V at maximal loads was 2.48 l (Table IV)

An increase in loading brings about a higher eathing frequency (Fig. 11) The average fre dency at maximal performances was 26/min (Table IV) The value is rather low compared to normal subjects, whose average rate is approximately 40/min. It is thus apparent that the exercise ventilation in these cardiacs was increased by raising the tidal volume more than by faster breathing.

Respiratory quotient

The respiratory gas exchange ratio (R) showed increasing values with increasing work loads, and the highest values were noted during loads requiring maximal oxygen uptake (Fig. 12). The mean maximal value for R was 1 11 (Table IV).

Table VI. *ECG changes during and/or after strenuous exercise as compared to the resting ECG*

ECG changes	No. of pats.
ST depression	3
T wave inversion	1
Both ST depression and inverted T wave	1
ST elevation	2
T wave converted to positive	7
Both ST elevation and T wave changed to positive	2
Intraventr. conduction disorder (broader QRS complex)	2
No changes from rest	3

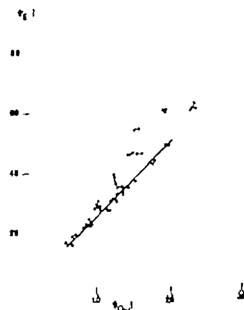


Fig 8. Pulmonary ventilation in relation to oxygen uptake. The normal regression line is drawn according to findings in healthy men of the same age (2).

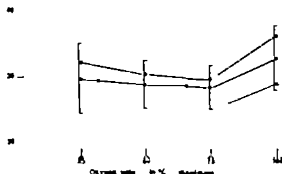


Fig. 9 Ventilation equivalent related to the percentage of maximal oxygen uptake compared to old men (5) and middle-aged healthy men (2). Vertical lines denote ±S.D for the cardiacs. O old men (76 y.)· ▲ middle-aged men (55 y.)· ● cardiacs (52 y.).

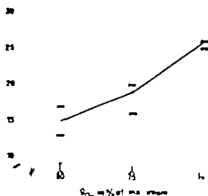


Fig. 11 Respiratory frequency related to increasing percentage of maximal oxygen uptake. — mean value

DISCUSSION

A striking feature in this study is the fact that post-myocardial infarction patients are able to perform heavy muscular exercise and tolerate maximal loads only 12 weeks after the onset of the infarction episode. No harmful consequences appeared during the xercise testing procedure. It is also noticeable that none of the patients, although heavily loaded, suffered from anginal pain during exercise in the laboratory environment. Subjects with xertional angina pectoris are stopped by chest pain, and consequently their physical performance capacity is usually smaller than in other coronary cardiacs. Some in estigators ha e shown that ventricular performance during exercise was less impaired in patients without anginal pain than in those with exertional angina (7 17)

The highest oxygen uptake was on average 25% lower than average maximal oxygen uptake for healthy men of comparable age body size, and with a habitual physical activity pattern charac teristic of sedentary people. Approximately half of the patients had values within the limits of normal variability (Fig. 2). It is likely that this group of male patients, who were engaged in daily work prior to the cardiac episode, were all within the normal range with regard to maximal

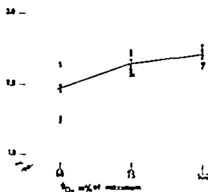


Fig. 10 Tidal volume related to increasing percentage of maximal oxygen uptake. — mean value

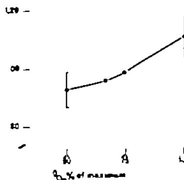


Fig. 12 Respiratory gas exchange ratio (*R*) related to increasing percentage of maximal oxygen uptake. Vertical lines denote ±S D.

oxygen uptake, and that their average was close to the average of the general population.

It may be stated, though this is a speculative assumption, that cardiac infarction brings about a reduction of the physical work capacity which can be estimated at about 25% 12 weeks after the acute episode. This level of fitness in the present cardiacs, averaging 52 years, corresponds to physical performance capacity found in healthy men aged 75 years (5)

This statement of the value of aerobic power in heart infarction patients agrees well with our earlier findings (3) and also with those of Kasser and Bruce (13) They reported an average maximal oxygen uptake of 26.5 ml/min/kg b.wt. in 28 healed infarction cardiacs who averaged 52 years of age. On the other hand, Kasch and Boyer (12) reported considerably lower values. They found an average of 19.9 ml/min/kg b.wt. in 11 men, aged 50 years, at least 1 year after the acute episode of heart infarction. Bolt et al. (6) tested 30 patients, 1–5 years in the post-infarction period, and found values of maximal oxygen uptake 36% lower than the average for healthy German people, and one-third of their patients were within the normal limits.

In a previous study (3) the maximal oxygen uptake was assessed in 24 cardiacs who had not returned to work 20 months after the acute infarction episode, and the average value was found to be 25.3 ml/min/kg b wt. or closely similar to the patients considered in this report. The reduction in physical work capacity seems thus to be established 12 weeks after the acute episode, and no further impairment takes place. To what extent a proper rehabilitation regimen can restore normality after an episode of heart infarction remains an open question in the absence of experimental data. The problem will be dealt with in a subsequent publication (4)

The hemodynamic response pattern does not deviate from the normal when the cardiacs perform submaximal exercise loads. A heart infarction reduces the amount of contractile elements of the myocardium, which may have several functional consequences, as pointed out in a previous report (3) This study confirms the earlier findings of a lower maximal heart rate in the post infarction period, the systolic arterial B.P also tended to be lower during heavy exercise, and did not reach the same high level as in normals. Heart rate increased in close linear relationship to metabolic rate up to the maximal level, while the systolic B P and the pulse pressure levelled off at submaximal intensities. The reduced maximal heart rate in coronary heart patients may be due to an impaired chronotropic response. The inability of the coronary patients to raise systemic systolic and pulse pressures during heavy exercise to the same extent as in normals may be due to a reduced myocardial contractility i.e. an impaired inotropic effect. More detailed analysis of the hemodynamic response to increasing and maximal loading requires cardiac catheterization, which was not performed as part of this work. In conclusion, the data to hand suggest that the heart's pumping capacity and its ability to raise the driving pressure are hampered by the myocardial infarction.

The oxygen pulse in maximal loading was on average about 20% lower than in normals. The oxygen pulse is a product of stroke volume and arteriovenous oxygen difference, and either of these factors may be affected by a myocardial infarction. Arteriovenous oxygen difference in submaximal exercise is found to be normal or slightly increased after cardiac infarction (8, 15, 16) It is therefore likely that the reduced oxygen pulse during maximal loading is related to a reduced stroke volume (10 15 16) which may also contribute to a lowering of the cardiac output in maximal exercise. Further an impaired cardiac efficiency during work may be inferred from the decreased maximal oxygen uptake and the normal heart volume. For a given heart size this brings about a lower oxygen uptake than in comparable normals.

In 13 out of 16 patients ECG changes occurred during the course of muscular exercise, indicating that coronary blood flow became insufficient. The changes appeared at an average relative load of 67% which fits in well with the observation in the previous study (3) where ECG alterations started at a relative load of 75% This is an important feature, because it suggests that the coronary blood flow is sufficient to cover the demand for oxygen in muscular exercise up to this load. A 67% relative load corresponds to an oxygen uptake of about 1.37 l/min in these subjects. Most jobs in industry require an oxygen uptake below this level, and consequently these heart infarction patients with a *sufficient* coronary

blood flow corresponding to an oxygen uptake of this value are probably sufficiently physically fit to sustain the strain of most jobs today

The observation of the reduced pulmonary efficiency (Figs. 8 and 9) in these post-infarction cardiacs is similar to that observed by Higgs et al. (9) and to our earlier findings (3) None of the patients presented symptoms or signs of heart failure or pulmonary congestion. The phenomenon is related to increased tidal volume which suggests that the consequence of a myocardial infarction is a change in pulmonary perfusion/ventilation rate. The same speculative conclusions have been drawn by others (10) but adequate data to support this statement are lacking.

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BIOCHEMICAL EVALUATION OF STANDARD TREATMENT WITH STREPTOKINASE IN ACUTE MYOCARDIAL INFARCTION

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Abstract. The biochemical effect of standard dosage of streptokinase (SK) in acute myocardial infarction (AMI) suggested to be less than 24 hours old has been evaluated. The trial was carried out in medical department of city hospital having no coronary unit. The SK dose was loading dose of 0.25 10^6 units, followed by 0.1 10^6/hour for 20 hours. Phenindione was given from the very beginning in the SK group (67 pats.) and in the control group (68 pats.). Special protocols were used. All therapy of any complication was standardized. The biochemical effect was evaluated by measuring the euglobulin clot lysis time (ELT), plasma thrombin time, fibrinogen, plasminogen, fibrinogen-fibrin breakdown products, α-2 macroglobulin, and α-1 antitrypsin, and the clinical effect by close ECG recordings and estimations of SGOT LDH and CPK. A persistent significant fibrinolytic effect was obtained in at least 85% of the patients for 20–4 hours. Plasminogen depletion was seen in around 15% generally lasting 1–2 days. Fibrinogen-fibrin breakdown products developed during the SK treatment. Increased amounts were seen in few other patients with thromboembolic complications or malignancy. Normal values were found in uncomplicated cases. The lethality was identical in the two groups, which were clinically comparable but not randomized. The frequency of bleeding complications was identical, but fewer thromboembolic cases were seen in the SK group, as illustrated by autopsy. The study showed that standard treatment can be performed without difficulties or serious complications, producing significant fibrinolytic activity in around 80–90% of the patients. The final evaluation of the possible effect of fibrinolytic therapy in AMI should be carried out in coronary units. Many well randomized patients are necessary.

Several papers dealing with the possible clinical effect of fibrinolytic therapy with streptokinase (SK) in acute coronary infarction (AMI) have been published (9 7 15 19 26, etc.) Single trials with urokinase-activated plasminogen (20) and fibrinolysin (25) have been performed. Very few trials have included biochemical data, and most of them have been conducted without control groups.

Three large scale control trials with SK ha e been reported. The German study (27) included 297 patients treated with SK f r 18 hours, followed by dicoumarol, and .51 control patients treated with anticoagulants only The patients were derived from medical departments of several hospitals. The time interval between onset of clinical symptoms and initiation of treatment was less than 12 hours. The lethality within the first 40 days of hospitalization was 14 1% in the SK group and 21% in the control group and, ex cluding patients dying within the first 24 hours of hospitalization, 8.7% and 16.1% respectively a difference which is significant. The SK treat ment followed two different schedules, but the results were not considered separately The biochemical effect of the SK treatment was not evaluated. The number of patients withdrawn from each group was not stated. The general treatment was not standardized.

Another European multicenter trial (2) consisted of 167 patients with myocardial infarction less than 72 hours old, of whom 84 received a loading dose of 1.2×10^6 units SK followed by 0.1×10^6 units/hour for 72 hours, followed by dicoumarol, whereas 83 patients received heparin and dicoumarol. No significant difference in le thality was demonstrated. In this study SK treat ment was compared to heparin treatment Loading dose was high, and the time interval from initia tion of symptoms to treatment was up to 72 hours. Few studies on fibrinolytic acti ity and blood coagulation were included.

Recently a third multicenter trial was published

Table I *Patients admitted for AMI during the investigation period*

	SK days (no. of pats.)	Control days (no. of pats.)
Admissions	170	173
Reason for exclusion		
> 24 h history	52	54
Contraindications	27	20
Immediate deaths	12	17
Wrong diagnoses (excluded later)	12	14
Excluded, all	103	105
Final material	67	68

(14) The SK dose differed in the various departments. Little information on the biochemical effect was given.

The aim of the present work was primarily to examine the biochemical effect obtained by giving a standard dose of SK (and phenindione) to patients suffering from AMI less than 24 hours old. The control group received phenindione. An approach to evaluation of the possible clinical effect was included. The study was carried out in one medical department and the general treatment was standardized. All patients with AMI were included in the analysis.

MATERIAL AND METHODS

Clinical material

The investigation period lasted from Jan. 1968 to May 1970. The medical department in city hospital received patients at any time, also very old and debile patients. The department had 3–5 transportable monitors, equipment for DC conversion, an emergency room with 2 monitors, but no continuous recording or alarm systems. Patients admitted on Mondays, Tuesdays and Wednesdays were treated with SK (and phenindione) and those admitted on Thursdays, Fridays and Saturdays with phenindione, if included in the trial. This division was made for practical reasons, but only after consulting the Danish Health Authorities (23), according to which the lethality among patients with AMI admitted in the first and in the second half of the week (Sundays and holidays excluded) to hospitals in Copenhagen is identical. The number admitted on SK days was 170, on control days 173 (Table I). Patients with a history exceeding 24 hours and patients with contraindications for treatment were excluded primarily. Several patients died before treatment with SK and/or anticoagulants could be established. Some patients were withdrawn later as the diagnoses appeared unproven. The final material consisted of 67 (SK days) and 68 (control days) patients.

Contraindications. Treatment with SK and/or phenindione was not carried out in patients with hypertension (systolic pressure > 200, diastolic pressure > 110 mmHg), acute pulmonary edema, pronounced renal or liver disease, ulcerative gastrointestinal disease, recent cerebral disease, surgical intervention less than three days previously, metastatic carcinoma, recent SK treatment, acute anticoagulant treatment, abnormal bleeding tendency or if treated with extracardiac massage.

Diagnosis, laboratory and clinical control. The diagnosis was in most cases based on typical history in all cases, distinct enzymic changes with elevation of SGOT, LDH and CPK, and on characteristic ECG patterns (except in 5 cases with BBB. ECG (including V +V +V extended to 12 leads if necessary), SGOT, LDH and CPK were examined every 6 hours during the first 24 hours, every 24 hours during the following 48 hours, daily on the following 4 days, and thereafter once a week. Fibrinogen-fibrin breakdown products were estimated in samples taken at intervals as indicated above. ELT and plasma thrombin time were estimated at the frequency indicated above when possible, whereas fibrinogen, plasminogen, α-1 antitrypsin and α-2 macroglobulin were estimated in samples taken before treatment and every 24 hours for three days, and thereafter once a week. ESR, Hb, leucocytes, urine, BP, serum creatinine, serum cholesterol, blood cholesterol, blood sugar, etc. were estimated in routine tests.

Special protocols were used and standard questions in them had to be answered at admission in order to obtain an equal evaluation of the patients. The protocols were used for daily reports, and specific standard questions

Table II. *Clinical comparability at admission*

	SK group	Control group
No. of pats.	67	68
Males	42	48
Females	25	20
Average age (y.)	65	62
Previous infarction	11	10
Previous angina pectoris	35	40
Previous hypertension	11	8
Diabetes mellitus (+ drugs)	9	6
Other atheroscl. disease	21	20
Average syst. pressure (mmHg)	149	149
Average diast. pressure (mmHg)	91	90
Average temperature (C°)	37.1	37.1
Average heart rate/min	87	84
Mainly anterior infarction	39	38
Mainly posterior infarction	24	22
Mainly transmural infarction	20	16
Shock	10	9
Arrhythmia	21	19
Heart failure	13	13
Interval from onset of symptoms to SK and/or phenindione (h)		
< 3	8	14
< 6	26	30
< 12	48	47
< 24	67	68

had to be answered. Treatment of all complications, such as cardiogenic shock, various forms of arrhythmia, heart failure etc., was standardized. Oxygen was not used routinely. The comparability of the two groups at admission is illustrated in Table II. The interval from the onset of clinical symptoms to the start of treatment with SK and or phenindione was little shorter in the control group than in the SK group.

Specific treatment. Streptokinase (Streptase®) was administered as follows: 0.25 10^6 units were given within 20–30 min, dissolved in 100 ml 5% glucose, followed by 0.15 10^6 units/hour (in glucose) for 20 hours. New solutions were prepared every 8 hours. Total amount of SK 3.25 10^6 units. Phenindione was administered from the very beginning, 50 mg 1 every 6 hours. The control group received glucose drop and phenindione as above.

Other therapy. The different drugs used in the two groups, according to the standard schemes, are listed in Table III. Sedative (Diazepam) was given to more patients in the control group.

Methods

Euglobulin clot lysis time (ELT) and *plasma thrombin time* were estimated as described elsewhere (11). These tests must be carried out immediately after drawing the blood. This could not always be done at the time requested, which explains the absence of results.

Plasminogen was measured according to the principles of Sherry et al. (28), *fibrinogen* by gravimetric method, *fibrinogen-fibrin breakdown products* (split products) by haemagglutination inhibition immunoassays according to the principles of Merskey et al. (21), *α-2 macroglobulin* and *α-1 antitrypsin* immunologically all as described elsewhere (10, 11, 3).

Thrombocyteaggregation was measured in "Born" aggregometer employing platelet-rich, citrated plasma at 37°C, under continuous stirring. Aggregation was produced by ADP. Details will be given elsewhere (6). Aggregation measurements were not included as routine, but were performed on selected patients before, during and after SK infusion or administration of the various drugs listed in Table III.

The coagulation tests were carried out in the Coagulation Laboratory whereas all other biochemical tests were performed in the Department of Clinical Chemistry of this hospital.

Table III *Other drugs used in the trial*

	SK group (*n*)	Control group (*n*)
Digoxin	23	25
Lidocaine	14	17
Procainamide	4	5
Isoproterenol	10	16
Metaraminol	3	4
Diuretics	23	24
Antibiotics	15	12
Diazepam	33	48

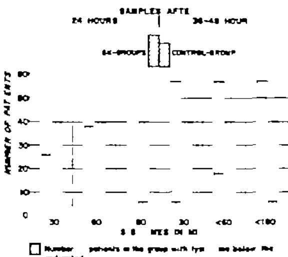


Fig. 1. Euglobulin clot lysis times (normal range 60–360 min).

RESULTS

Biochemical results

Especially the 24-hour samples illustrate the biochemical activity and will be discussed in detail.

Euglobulin clot lysis time (Fig. 1) 24 hours after initiation of SK treatment was shorter than 30 min in 40% of the patients, shorter than 60 min (lower limit of normal range) in nearly 80% and shorter than 180 min in all. More than 60% of the patients in the control group had ELT above 360 min, the upper limit of the normal range. After 36–48 hours all patients in the SK group still had an ELT within or below the normal range, whereas none in the control group had ELT below the normal range. After three days two-thirds of the patients in the SK group had ELT below 180 min, whereas one half of the control group had ELT longer than

Table IV *Plasminogen concentrations in 50 consecutive patients in 24-hour samples after SK*

Plasminogen conc. ( )	No. of pats
40	49*
< 30	47
20	43
10	28 (7 dead)
0	7 (4 dead)

One patient > 50 SK ran subcutaneously

Table V *Plasma thrombin times 24 and 36–48 hours after SK and phenindione*

(normal range 12 17 sec)

Clotting time (sec)	24 hours SK group	24 hours Control group	36–48 hours SK group	36–48 hours Control group
< 15	1	28	18	31
15–17	4	14	20	5
18–22	12	—	17	2
23–29	17	—	4	—
30–59	15	—	—	—
≥ 60	2	—	—	—
Total	51	42	59	38

360 min. After one week around 50% of all patients had ELT exceeding 360 min, i.e. very low activator activity

Plasminogen concentration (Table IV) dropped in all, and was below 10% in nearly 60% of the first fifty patients in the 24-hour samples. A zero concentration was found in around 15% The plasminogen increased in general to > 50% within

Table VI *Fibrinogen concentrations in SK and control groups*

Range (mg)	At admission SK group	At admission Control group	24 hour samples SK group	24 hour samples Control group
0– 49	—	—	2	—
50– 99	—	—	3	—
100–199	1	—	13	—
200–299	4	5	20	3
300–399	17	15	7	9
400–499	19	22	5	16
500–599	7	6	1	13
600–699	3	4	—	7
700–799	1	1	—	4
800–899	1	1	—	—
Total	53	54	51	52

Mean values at various times	SK group (mg)	SK group (n)	Control group (mg%)	Control group ( )
At admission	434	53	429	54
After 24 hours	247	51	502	52
After 1 week	500	38	568	43
After 2 weeks	502	34	550	32
After 3 weeks	463	29	541	24
After 4 weeks	438	20	525	18

Table VII *Split products (in µg/ml serum) (+ EACA) before and after SK (normal range < 20 µg/ml)*

Normal values were found repeatedly in the control group during all 4 weeks, except in a few cases, see text

	<20	20–40	40–100	100–200	200–400	400–1200	Total
Before SK	56		2				58
After 24 hours	1		4	23	16	10	54
After 48 hours	4	7	22	13	6	1	53
After 72 hours	15	13	11	2	1		42
After 1 week	18	2	3				23
After 2 weeks	28	6					34
After 3 weeks	28	1					29
After 4 weeks	28						28

SK ran subcutaneously

2–3 days but often did not reach the initial level until after 3–4 weeks. In one patient it remained very low This patient died. At autopsy a pulmonary embolus more than one week old was found.

Plasma thrombin times (Table V) were significantly longer than normal in around 90% of the 24-hour samples, and in around 30% after 36-48 hours. After three days all thrombin times were normal or shorter than normal. Most patients in the control group had clotting times within the normal range, often even lower

Fibrinogen concentration (Table VI) *dropped* in all during the fibrinolytic therapy (except in the patient who had SK subcutaneously) and significantly lower values were found after 24 hours compared to the controls. The difference between the two groups decreased, but persisted for at least 4 weeks. Highest values were found in both groups between the first and second week.

Concentrations of split products in serum were within the normal range in nearly all patients at admission. A distinct increase was found during treatment with SK (Table VII) persisting for several days. Normal values were often not found until after one or two weeks. Increased concentrations were found at admission in two patients in both groups. One in each group died and exte si e mural thrombi were found in the heart. Two survivors had clinically pulmonary infarctions. Three more in the control group had later increased alues. One died several thromboemboli being found at autopsy whereas the two survivors suffered clinically from phlebothrombosis in a

lower extremity One other in each group had permanently high values and appeared to suffer from metastatic carcinoma. Increased concentrations of split products were otherwise not found in AMI.

α-1 antitrypsin increased significantly after SK infusion (Fig. 2), often to a twofold concentration, in general for around weeks. A slight increase was also seen in the control group. The for the whole group was very large.

α-2 macroglobulin (Fig. 2) decreased significantly in concentrations after SK infusion and remained generally low for 2–3 weeks. A slight increase was seen in the control group. The range was very large. Extremely high concentrations were found in some patients dying with wide d thromboemboli and in patients with car

P–P%. A faster decrease in P–P% was seen the SK group (Table VIII) which did not higher dosages of phenindione. The P–P% was lower than 20 in nearly 80% of the 24-hour

Thrombocytaggregation (Fig. 3) was generally

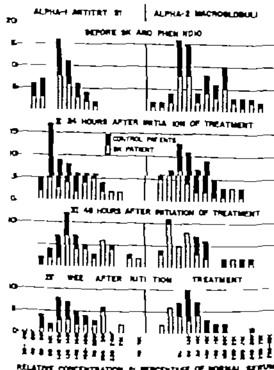


Fig. 2 Relative concentrations of α-1 antitrypsin and α-2 macroglobulin

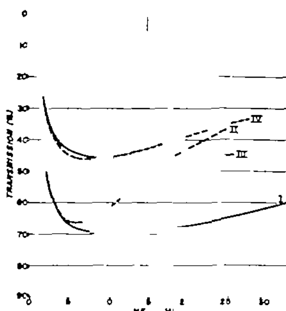


Fig 3 Platelet aggregation after addition of ADP to citrated platelet-rich plasma (final conc. 1 5 mM/l at 37°C). I = before, II = 10 min after infusion of Diazepam (10 mg i. .), III = before, IV = after infusion of SK (0 5 10⁶ units). In some patients SK rather increased aggregation and no disaggregation occurred.

but not always inhibited during SK infusion, whereas a drug such as Diazepam always accelerated disaggregation. Other drugs exhibited a less distinct effect and will be discussed elsewhere (6)

Other results. The ECG examinations and the enzyme patterns will not be discussed in this paper These laboratory controls were primarily performed for verification of the diagnosis and will be published separately

Clinical results

The clinical results and the lethality are summarized in Table IX. The frequency of early

Table VIII. *P–P% for first 50 patients in each group 24 and 36 hours ft onset of treatment*

%	24 hours SK group ( )	24 hours Control group (n)	36 hours SK group ( )	36 hours Control group (n)
20	34	23	44	31
21 30	15	15	6	11
30	1	12	0	8

Table IX. *Clinical results*

	SK group (n)	Control group (n)
Total material	67	68
Survivors	51	48
Deaths	16	20
Early		
24 h	4	8
24–72 h	7	2
Late		
< 40 d.	5	8
> 40 d.	—	2
Lethality	(%)	(%)
Overall	23.1	29 4
Excluding first 24 h of hospitalization	17.9	17 7

No significant differences.

deaths was the same in the two groups, and so were the clinical diagnoses at death. Secondary infarctions were more frequent in the control group (Table X)

Autopsy AMI was found in all autopsy cases (13 of 16 deaths in the SK group and 19 of 20 in the control group) The frequency of thrombi was significantly higher in the control group, whether coronary thrombi, mural thrombi or extracardiac thromboembolic cases (Table XI) The frequency of rupture was considered identical. The fibrinolytic activity was not more pronounced in patients with rupture Secondary infarctions due to fresh thrombi were seen in two cases in the control group. Cerebral hemorrhages or hemorrhagic cerebral infarctions were not seen.

Complications (Table XII) Chills after SK occurred in several patients, but were not pronounced. Rash was seen in one, heavy back pain in two and the SK infusion was stopped in one of these. Short lasting asystolia occurred in two: both survived One received isoproterenol, whereas the other was treated with extracardiac massage, and the SK infusion was stopped. A small drop in BP was seen in some patients if the loading dose was given within 20 min, but very seldom and insignificant if the infusion time was above 30 min Macroscopic bleedings were especially seen on the second day A patient with P–P% 6, and a patient with bleeding from the bladder had blood transfusion. Bacteriemic complications were found in two in the SK group both survived. Early pulmonary infiltrations were found in ten in the SK group, in six in the control group (X-ray examinations were performed in all) Three patients in the control group had macroscopic hemorrhages, and one received a transfusion.

Lethality in excluded patients. The lethality in patients admitted on SK days or on control days, but excluded, was calculated in order to evaluate a possible unequal distribution in the various groups. The lethality was identical in both groups.

DISCUSSION

The potential effect of thrombolysis in treatment of thrombotic coronary occlusion has already been a matter of vivid discussion.

The frequency of thrombi in coronary arteries found at autopsy in AMI has varied from very

Table X. *Clinical diagnosis at death*

	Early		Late		Total	
	SK group (n)	Control group (n)	SK group (n)	Control group (n)	SK group ( )	Control group (n)
Asystolia	3	2	—	—	3	2
Rupture with tamponade	3	4	2	—	5	4
Arrhythmia	1+1	2	2	4	4	6
Heart failure	2	2	1	2	3	4
Reinfarction	—	—	—	2+1	—	3
Pulmonary embolus	—	—	—	1	—	1
Ileus	1	—	—	—	1	—
	11	10	5	10	16	20

Not verified by autopsy

low values to as much as 96% (22). Some of these thrombi are suggested to have been secondary to the infarction (29). Even if the frequency is that high, and even if the fibrinolytic activity might reach the thrombus formation, these platelet-rich thrombi are not an ideal substrate for fibrinolytic therapy (13). Furthermore, the myocardial damage developed before the fibrinolytic activity can lyse the actual thrombus. Thrombolytic therapy may on the other hand, digest some of the fibrin-rich thrombi present in the ischemic surroundings of the myocardial infarction (12, 13) and thereby inhibit progression, restore collateral circulation and favor healing processes.

The reduction of fibrinogen in circulating blood might reduce the viscosity and improve circulation, and the interference of fibrinogen breakdown products with blood clotting (8) and platelet aggregation (18) might delay or even inhibit further thrombus formation inside and outside the heart.

The lethality in AMI has no doubt been reduced in recent years due to better observation, especially in coronary units, resulting in immediate and improved treatment of complications, which especially threaten patients, such as arrhythmias, heart failure, cardiogenic shock etc. If fibrinolytic therapy is to reduce the lethality further it should be conducted in coronary unit.

In materials published up to now fibrinolytic therapy has taken place predominantly in medical departments. The general treatment of the very important complications has not been standardized, at least not according to the publications. Very few papers have defined the biochemical effect of the fibrinolytic therapy.

The aim of the present study was primarily to evaluate the biochemical effect of a standard dose of streptokinase given to patients with AMI suggested to be less than 24 hours old. It is uncertain which criteria one should define as ideal and satisfactory in fibrinolytic therapy of AMI, but it is nevertheless important to describe the methods used. It was possible with our standard scheme to obtain a significant fibrinolytic activity in circulating blood lasting for at least 4 hours in around 85% of the patients, as illustrated by increase in plasminogen activator activity, decrease in plasminogen and fibrinogen, increase in fibrinogen-fibrin breakdown products and by prolongation of plasma thrombin time. A distinct correlation between these parameters existed. The increase in fibrinolytic activity contrasted markedly with the decrease in activity normally seen after AMI, as also described by others (4, 5). The biochemical changes produced by SK agree well with those recently described by Hirsh et al. (17) and Amery et al. (1). They used the same loading dose, but a continuous dose of 0.1×10^6 units/hour for 24 hours. The higher loading dose (1.2×10^6 units) previously used by Amery et al. (1) is suggested to be too high. The plasminogen

Table XI. *Findings at autopsy*

	SK group (*n*)	Control group (*n*)
AMI	13	19
Coronary thrombus	5	10
Mural thrombus	2	5
Macroscopic myocardial hemorrhage	4	8
Rupture with tamponade	5	4
Pulmonary embolus	1	1
Other thromboemboli	2	4
Reinfarction	—	3
Reinfarction with fresh thrombus	—	2

More than week old.

Table XII. *Complications*

Streptokinase	
Allergic	
Heavy back pain	2
Asystolia (+ resusc. in 1)	2
Drop in BP	several
Bleedings (2nd day)	
Nose (P–P% 16)	1
Nose (P–P% 6)	1
Urine (P–P% 17)	1
Bacteriemic?	
Bacteriemia	2
Bronchopneumonia (6 in control group)	10
Cystopyelitis	8
Phenindione	
Hematemesis (P–P% 14)	1
Urine (P–P% 20)	1
Hematoma (P–P% 7)[a]	1

SK infusion not stopped in time.
[a] + acetylsalicylic acid.

concentration remained below 5% for more than 2 weeks in one patient, and a pulmonary embolus more than a week old was found at autopsy The possible relationship between the long-lasting depletion and the development of the pulmonary embolism is uncertain, but except for this case significantly fewer thrombotic manifestations were seen in the SK group at autopsy

The drop in α-2 macroglobulin generally seen after SK infusion, also described by Niléhn and Ganrot (24) is presumably due to its binding to plasmin, and the sustained elevation of split products is possibly due to the reversibility of this binding. Very high concentrations of α-2 macroglobulin were seen in some patients before treatment and in two, who died, extensive thrombus *formations were seen at autopsy Also the in*crease in α-1 antitrypsin after SK has been described previously (4) but an increase was also seen in the control group. The fibrinolytic activity obtained could in general not be correlated to the concentrations of the two antiplasmins. Furthermore the 10–15% who did not react satisfactorily did not have higher concentrations of antibodies against SK.

The combination of streptokinase and phenindione made it possible to omit heparin treatment, as the P–P% generally was within the accepted [illegible] when the streptokinase treatment was stop-[illegible] *due to the fact that also clotting factors,* [illegible]sured by this method, are digested by plas-[illegible]n.

Bleeding episodes or cardiac ruptures were not more frequent or extensive in the SK group. Unpleasant complications such as asystolia did occur if the infusion time of the dose was less than 30 min. No patient had puncture of an artery

The experience that Diazepam, which was administered to many patients, especially on the first days after admission accelerates platelet disaggregation significantly—a hitherto unpublished finding (6)—stresses the importance of standardizing all therapy Reports on the influence of fibrinogen breakdown products on platelet aggregation are conflicting. Some authors describe an inhibitory and others an accelerating effect (18 30 31) One should remember that antigen-antibody complexes accelerate platelet aggregation. A combination of fibrinolytic and antiaggregation therapy might be worthwhile trying.

ACKNOWLEDGEMENTS

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DIABETES MELLITUS AND DEFECTIVE PITUITARY RESERVE CAPACITY

A Case Report

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Abstract A case of diabetes mellitus with an incomplete Sheehan syndrome, consisting of an almost complete loss of pituitary reserve capacity while the basal pituitary function seemed to remain unaffected, is presented. Menstruations were regular and basal plasma levels of cortisol, FSH, LH, and PBI were normal. There were no characteristic symptoms except for an extreme insulin sensitivity so without the diabetes the patient's partial pituitary insufficiency may well have remained undetected. The present case illustrates that severe pituitary damage can take place without characteristic symptoms, causing a potentially dangerous state for the patient in a stressful situation, e.g. an operation. Some of the non-specific symptoms in the present case (tiredness, weight loss) were relieved by substitution therapy

In 1935 Lyall and Innes (8) described the first case of a human counterpart to the Houssay preparation in dogs, namely a diabetes mellitus combined with an insufficiency of the anterior pituitary gland. Since then several cases of this syndrome have been described, and a far greater number have been produced surgically by hypophysectomy in an attempt to avoid the progress of an imminent diabetic retinopathy

The symptoms are usually those characteristic of pituitary insufficiency and in addition an extreme insulin sensitivity in several cases reported to be fatal.

The present case illustrates that considerable functional loss of the pituitary gland can take place, causing only non-specific symptoms, which per se were not characteristic enough to arouse suspicion about the diagnosis. Instead, it was the patient's pre-existing diabetes mellitus that, on account of a pronounced insulin sensitivity after delivery initiated the laboratory investigation which established the diagnosis of pituitary insufficiency

Especially in diabetics the diagnosis of a partial pituitary insufficiency is important, as even isolated losses of pituitary hormones have been reported to produce dangerous hypoglycaemia (17) and the patient may suffer an increased risk, e.g. at an operation, unless the condition is known and extra corticosteroids can be administered.

CASE REPORT

The patient is a woman, now aged 30. At the age of 9 diabetes mellitus was diagnosed and insulin therapy started. During recent years the dose has been about 80 U daily

At the age of 26 her first pregnancy was free from complications and she gave birth to a healthy child. During this pregnancy microaneurysms were found for the first time. After the pregnancy good control of her diabetes was again obtained by 80 U of insulin daily

The next pregnancy at the age of 28, terminated in the 30th week with an unexpected, premature delivery in spite of good control of blood glucose and absence of signs of toxicosis. The child weighed 900 g and survived for 3 weeks, suffering from oesophageal atresia (for which a successful operation was performed) and bilateral renal agenesis.

During the delivery a moderate hypoglycaemia could be counteracted by glucose orally. There was no extensive bleeding and no signs of shock. In view of the bad condition of the child, the patient received 20 mg of ethinyloestradiol and 10 mg of norethisterone to prevent lactation.

The requirement of insulin was about 100 U daily before delivery after the delivery only 4–6 U daily and has not changed since then.

On the day after delivery icterus and itching was observed. The serum bilirubin level was 4 mg/100 ml, GOT and GPT 150–200 U, and alkaline phosphatase 18 U. A diagnosis of pregnancy icterus was made. After 3 weeks of corticosteroid therapy (during which the insulin requirement was about 20 U daily) subjective symptoms had disappeared and laboratory tests were normal. The

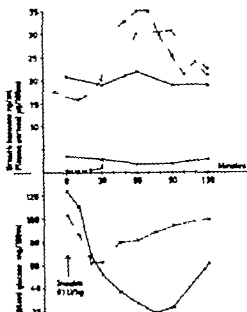


Fig. 1. Response of growth hormone (O) and plasma cortisol ( ) in the patient (—) compared to mean normal responses ( ) during hypoglycaemia induced by insulin, 0.1 U kg b.wt. The mean normal response of plasma cortisol according to Arner et al. (1), that of growth hormone to Greenwood et al. (5). All tests, in patient and in controls, were started at about 10 a.m.

corticosteroid therapy was withdrawn. Later laboratory tests for liver function including bromsulphthalein retention have been normal.

Within 2 months after this delivery menstruations returned and have been regular and normal since then. The patient is not taking oral contraceptives. PBI, triiodothyronine uptake test and plasma cortisol at 8 and 22 h were normal on repeated occasions.

The patient has suffered frequent insulin reactions, of moderate degree but often lasting for several hours, in spite of the low dosage of insulin used after the delivery. However, when complete withdrawal of insulin was tried, the blood glucose increased to 485 mg 100 ml and a slight ketonuria appeared.

On admission to Medical Department A, University Hospital, Lund, 2 years after the delivery the patient subjective complaints were the frequent insulin reactions, a non-specific tiredness, and the fact that her body weight had decreased to 48 kg compared to the usual 54–55 kg before the pregnancy.

The result of physical examination was normal. The BP was 105/70 mmHg. The body hair was normal. Breasts and gynaecological examination were normal.

Routine laboratory tests including tests for liver function were normal except for a slight anaemia with Hb concentration of 11.5 g/100 ml. The sella turcica was normal on X-ray examination.

The decreased requirement of insulin induced the following investigation of pituitary function.

Growth hormone

Intravenous insulin tolerance test, 0.1 U/kg b.wt., led to a hypoglycaemia with lowest blood glucose value of 19 mg/100 ml but no increase of plasma growth hormone concentration (radioimmunoassay) over control values (Fig. 1). Normally a hypoglycaemia of this degree should induce an increase of at least 13.7 ng/ml (5). During the infusion of arginine (0.5 g/kg for 30 min) no increment of growth hormone (radioimmunoassay), determined at 30-min intervals for 3 hours, was obtained. (Radioimmunoassays were performed by Dr J. Thorell, Allmänna sjukhuset, Malmö.) However, this result should be interpreted with caution as Merimee et al. (9) have reported an impaired growth hormone response to arginine in diabetics.

Gonadotropin

Repeated determinations of urinary gonadotropins by the mouse ovarian weight assay showed 10 U in all assays, which is within normal limits for sex and age.

Basal temperature followed for 2 months indicated ovulatory menstrual cycles.

Serum FSH was between 0.8 and ~5 ng/ml, serum LH 3.0 ng/ml, which are normal levels for both hormones. (Serum FSH and serum LH were determined (radioimmunoassay) by D. L. Wide, Akademiska sjukhuset, Uppsala.)

Corticotropin

Fluorimetric determination of plasma cortisol on repeated occasions showed morning values of 18, 19, 16 and 1 μg/100 ml respectively and night values of 10 and 11 μg/100 ml, which is well within normal range of the method used. The blood glucose was between 100 and 200 mg/100 ml at all these samplings. ACTH, 30 U i.v. increased the plasma cortisol concentration from 18 to 48 μg/100 ml in 90 min, which is normal response. However during insulin-induced hypoglycaemia (1) there was no significant increase of plasma cortisol concentration (Fig. 1).

Metyrapone, 750 mg 6, did not increase the urinary excretion of 17-hydroxycorticosteroids. The control level, 1.0 mg 24 h, was unexpectedly low with respect to the normal basal plasma cortisol level, and the excretion of 17-ketosteroids was also low 1.5 mg/24 h. The biological half-life of cortisol, investigated by the administration of ^{14}C-cortisol (determined by Dr S. Laurell, Department of Clinical Chemistry University Hospital, Lund) was 115 min (normal range 78 ± 4 min (17))

These investigations indicate a sufficient basal adrenocortical activity which is aided by slow metabolism of cortisol but complete loss of the pituitary corticotropin reserve.

Thyrotropin

The plasma PBI was 4.5 and 5.2 μg 100 ml respectively, and the triiodothyronine uptake test 84 and 82% of the mean value obtained from normal control material. The fasting plasma cholesterol was 220 mg 100 ml. All values mentioned above were within normal limits according to the methods used for determination. However, the BMR was ~36%.

Stimulation with thyrotropin-releasing hormone, the tripeptide pyroglutamate-histidine-proline amide (Kabi, Stockholm), 1 000 μg i.v. produced a maximal increase of plasma TSH (determined (radioimmunoassay) by Dr L. Wide, Akademiska sjukhuset, Uppsala) from 1.5 to 4.8 μU/ml after 20 min. This is a subnormal response to TRH according to Hershman and Pittman (6).

The ^{131}I uptake during 2 h was 10%, after 24 h it was 24%, with an excretion during the first 24 h of 50% of the dose administered. These values are normal, but near the lower limit of the normal range. After 3 days with administration of 10 U of thyrotropin daily the 2 h uptake had increased to 26%.

These investigations indicate a reduced ability to increase the release of thyrotropin from the pituitary gland. The response of the thyroid gland to thyrotropin was normal.

Retinopathy

During the first pregnancy microaneurysms were found bilaterally for the first time. The retinal state remained stationary until the second pregnancy. Two years later, however, an improvement of the retinal state was registered with almost complete regress of microaneurysms and no other signs of retinopathy.

DISCUSSION

In spite of only moderate and non-specific subjective symptoms, an extensive loss of pituitary reserve capacity was diagnosed in the present case. The basal function was, however, apparently retained to such an extent that the characteristic symptoms of pituitary insufficiency did not appear. Thus menstruations were regular and basal plasma cortisol, growth hormone, FSH, LH and PBI levels fell within normal range.

A partial pituitary insufficiency affecting primarily the reserve capacity may thus be difficult to detect in the absence of characteristic symptoms and affection of basal hormone production, as only tests of pituitary reserve function may reveal the condition.

The order of falling out with increasing injury to the pituitary gland has been stated by Rabkin and Frantz (14) and Fraser (3) to be as follows: growth hormone, gonadotropins, corticotropin, thyrotropin. However, the present case illustrates that the reserve capacity for several hormones may be severely reduced before any of them is affected enough to cause any of the usual characteristic symptoms of pituitary insufficiency. Apparently such a condition may easily be overlooked, and we do not know how many patients with such incomplete pituitary insufficiency remain undetected and suffer an increased risk in a stressful situation, e.g. an operation.

In the present case the diagnosis was suspected due to the frequent long-standing hypoglycaemias and the low doses of insulin in the treatment of the patient's diabetes mellitus. This is easy to understand as she lacks two important mechanisms of defense against hypoglycaemia, namely the ability to release extra growth hormone and corticotropin. Without the diabetes and insulin treatment the patient's pituitary insufficiency may well have remained undetected.

As the increased insulin sensitivity appeared promptly after delivery, it is reasonable to regard the condition as an incomplete Sheehan's syndrome. There was no extensive bleeding during the delivery and no signs of hypovolaemic shock, but such symptoms are not always observed preceding even a complete Sheehan's syndrome, and were absent in 6 cases in a material of 57 patients reported by Murdoch (10). The aetiological significance of hypoglycaemia and pregnancy jaundice for the damage to the pituitary gland is by no means clear. However, pituitary necrosis has been reported to be more common in diabetics than in others in an autopsy material (2), and Frey (4) considered a diabetic angiopathy to be the cause. It is possible that the diabetic's pituitary gland is more vulnerable and may suffer damage from noxious influences that do not damage a normal pituitary gland. Autopsy material has shown that rather extensive necrosis of the pituitary gland may take place before major symptoms of deficiency occur (15).

A pituitary insufficiency secondary to a hypothalamic damage remains as a possibility, but in view of the normal diurnal variation of plasma cortisol, a damage at the pituitary level seems more probable in the present case.

The regress of the patient's discrete diabetic retinopathy is in accordance with experiences from cases with more complete pituitary insufficiency (11, 12). Diabetic retinopathy has been ascribed to growth hormone (7), which is in accordance with the regress noted in the present case with a deficiency of growth hormone. However, this mechanism has been denied by others (13), and the question of diabetic retinopathy is still far from clear.

The detection of a pituitary insufficiency, even a partial one, is important insofar as it enables

(contd. 191)

the relief of symptoms, which, however non-specific, may be very disturbing to the patient. In case of an acute stressful situation, e g. an operation, these patients would also suffer an increased risk unless extra corticosteroids are supplied.

In the present case substitution with methyltestosterone, 5 mg daily and fluorhydrocortisone 0.1 mg daily led to increased well-being and weight increase. With respect to the normal basal plasma cortisol a continuous corticosteroid substitution was neither considered necessary nor expected to solve any problems. Instead, the patient was instructed to take 2.5–5 mg of prednisolone together with glucose in case of an insulin reaction. With this regimen the insulin reactions have been less durable and easier for the patient to manage

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MYCOPLASMA IN URINE COLLECTED BY SUPRAPUBIC ASPIRATION

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Abstract. The present study indicates that mycoplasmas may invade the urinary tract above the urethra. From 87 patients with signs of urinary tract infections voided urine, as well as urine collected by suprapubic aspiration from the bladder, has been cultured for *Mycoplasma*. Besides culture these specimens and voided urine specimens from an additional 190 patients were examined with a screening test for T-mycoplasmas which utilizes the ability of these organisms to metabolize urea. Among the 87 patients T-mycoplasmas were cultured from voided urine in 18 cases and *Mycoplasma hominis* in 9 cases. In 10 of the 18 patients (56%) T-mycoplasmas were isolated also from urine collected by bladder puncture. In 2 of these 10 patients the cultures from the aspirated urine also yielded growth of *M. hominis*. T-mycoplasmas were in no instance found on culture unless the screening test indicated that urea had been metabolized with the production of ammonia.

Mycoplasmas can frequently be isolated from voided urine specimens, where they generally seem to represent a contamination from the urethra or the periurethral tissues. The possibility of mycoplasmas as causative agents in non-gonococcal urethritis has been the subject of numerous studies, but an aetiological role of *Mycoplasma* in this condition has not been established (2, 3). Witzleb et al. (13) reported the isolation of *Mycoplasma* from urine collected by bladder puncture in 4 patients with urinary tract infections.

In the present study cultures for *Mycoplasma* from urine collected by percutaneous suprapubic aspiration (SPA) from the urinary bladder and from voided urine from the same individuals were performed in a series of 87 patients with urinary tract infections. The study includes an evaluation of a screening test for the demonstration of T-mycoplasmas in urine specimens. The test is based upon the ability of T-mycoplasmas to hydrolyse urea with the production of ammonia (10, 11).

MATERIAL AND METHODS

The 87 cases from which cultures were performed from urine specimens collected by SPA, as well as from voided urine, had been participants in a screening survey for bacteriuria and proteinuria including about 3 500 persons between 18 and 73 years of age. The 87 cases, 80 women and 7 men, were selected at random among about two hundred subjects who underwent further investigations, including suprapubic bladder puncture, because of findings suggesting urinary tract infections. Thus no selection for SPA was made according to findings of mycoplasmas in voided urine. Forty-two of the 87 cases had the diagnosis of chronic pyelonephritis. Of the remaining 45 cases 28 had significant bacteriuria as defined by Kass (4). In these 28 cases there were normal X-ray findings, and the laboratory data did not suggest impairment of kidney function. This was also valid for 17 patients who did not have significant bacteriuria but who manifested dysuria, pollakisuria and pyuria, in some cases combined with proteinuria and haematuria. No antimicrobial treatment had been given to any of the 87 cases for at least weeks prior to the sampling. The number of cases within the 10-year groups 15–24, and so on, were 6, 18, 10, 16, 23 and 14 respectively. The youngest patient was 18 and the oldest 73 years old.

Sampling methods

Using a 8 cm long disposable needle, diameter 0.8 mm, the urinary bladder was punctured in the midline 1 to 2 cm above the symphysis pubis. Sampling was done 6 to 10 hours after previous voiding. Immediately after SPA a mid-stream portion of the voided urine was collected. Periurethral cleansing after SPA and before voiding was done with dry sterile cotton compresses. The specimens were immediately chilled and the cultures were set up within 4 hours.

Culture of Mycoplasma

The procedures for isolation and identification of T-mycoplasmas, *Mycoplasma hominis* and *Mycoplasma fermentans* have been described earlier (7, 8). The urine specimens were inoculated on agar plates using a calibrated loop technique (8). The plates were incubated at 37°C in an atmosphere of 90% N_2 and 10% CO_2. The number

Table I *Isolation of T-mycoplasmas and Mycoplasma hominis in urine specimens collected by SPA and in voided urines from the same patients* ($n = 87$)

	Urine specimen	No. of specimens yielding growth	No. of CFU/ml of urine		
			$< 10^3$	10^3–10^4	$> 10^4$
T-mycoplasmas	Bladder	10	6	2	2
	Voided	18	13	3	2
M hominis	Bladder	2	2	0	0
	Voided	9	5	3	1

of colonies was counted with the aid of a stereomicroscope after 3 and 7 days.

A 0.1 ml portion of urine was also inoculated into a liquid medium for the demonstration of the urea-splitting ability of T-mycoplasmas. This medium contained the same nutritive components as those used for the isolation of T-mycoplasmas, but also urea and phenol red (8).

Isolation of bacteria

Quantitative bacterial cultures were made by the same calibrated loop technique as mentioned above (8). Inoculation was done on blood agar plates incubated aerobically at 37°C and read after 16 to 20 hours, as well as after another 2 days.

Screening for T-mycoplasmas

Apart from being cultured for T-mycoplasmas, voided urine samples and urine samples collected by SPA from the last 64 of the 87 cases presented above were inoculated into the urease colour test tubes.

From an additional 190 cases voided urine specimens were examined both by culture and by inoculation into the urease colour test tubes. These cases were participants in the health survey mentioned above or patients at the renal clinic. Of the patients from the renal clinic, 70 females and 23 males, 51 had the clinical diagnosis of chronic pyelonephritis, 10 chronic glomerulonephritis, whereas the remaining 3 cases had a variety of diagnoses (urinary tract infection, etc.). All the other 97 cases, 73 females and 24 males from the health survey had pyuria, but none of them had significant bacteriuria. Pyuria in the women was considered when 10 or more WBC per high power field (HPF) (× 320) were present in the sediment of 10 ml urine centrifuged at 5 000 rpm for 5 min. The corresponding figure for the men was 5 or more WBC/HPF.

RESULTS

Occurrence of Mycoplasma in urine collected by SPA and in voided urine from the same patients

The isolation rates of T mycoplasmas and *M hominis* in SPA urine and voided urine of the same individuals are shown in Table I. T-mycoplasmas and *M hominis* were never recovered from SPA urine unless the organisms were cultured also from voided urine of the individual. *M fermentans* was isolated from voided urine but not SPA urine in one case. The urine specimens collected by SPA yielded growth of T mycoplasmas in 10 of the 18 cases in which these organisms were also recovered from voided urine. The number of colony-forming units (CFU) of T-mycoplasmas per ml of voided and SPA urine was of the same magnitude in all but one of the 10 cases. In this case there was sparse growth of T-mycoplasmas ($< 10^3$ CFU/ml) in the specimen collected by SPA, whereas the voided urine yielded moderate growth (10^3–10^5 CFU/ml). In 5 of the 10 SPA urine specimens with growth of T-mycoplasmas there was no accompanying growth of bacteria. In the remaining 5 cases the SPA urine, as well as the voided urine yielded concomitant, heavy growth of bacteria ($> 10^5$ organisms per ml of urine) *Escherichia coli* in 3 cases, *Staphylococcus epidermidis* and coliform rods in one case each. In the last-mentioned case with growth of coliform rods, and in one of the cases with no growth of bacteria, cultures from SPA urine yielded sparse growth of *M hominis*; this organism was also found in voided urine of these cases.

Five of the cases in which *Mycoplasma* was isolated from urine collected by SPA had chronic pyelonephritis, and in 2 additional cases this disease was probable. The remaining 3 cases had significant bacteriuria, but there was no evidence of pyelonephritis. Three of these 10 cases are presented below as case reports.

It may be mentioned that *M hominis* was found in three instances to grow also on the ordinary blood agar plates used in the bacterial examination. In these instances very tiny transparent colonies were found after 72 hours of incubation. The identification of the colonies as *M hominis* in these cases was done by subcultures and subsequent typing on *Mycoplasma* medium.

In 35 of the 77 cases in which no growth of *Mycoplasma* was obtained from SPA urine, the bacterial cultures yielded no growth from urine collected by SPA or from voided urine. In SPA urine, as well as in voided urine from 39 cases there was heavy growth of bacteria. *E coli* in 32 cases, coliform rods in 4 and *Streptococcus*

Table II *Screening of urine specimens for T-mycoplasmas by the "urease colour test"*

T+ = T-mycoplasmas found on solid medium, T− = T-mycoplasmas not found on solid medium

Urine specimen	No. of specimens tested	Urease activity T+	Urease activity T−	No urease activity T+	No urease activity T−	Non-conclusive tests T+	Non-conclusive tests T−
Bladder	64	7	1	0	53	0	3
Voided from the same cases	64	14	4	0	42	0	4
Voided from cases not submitted to SPA	190	61	12	0	92	0	25
Total	318	82	17	0	187	0	32

faecalis, *Proteus mirabilis* and *Pseudomonas aeruginosa* in 1 case each. In 2 cases both SPA urine and voided urine yielded moderate growth of *E. coli* and *Streptococcus faecalis* respectively and in 1 case the cultures showed only sparse growth of *E. coli*.

Screening for T-mycoplasmas by the demonstration of urease activity

Altogether 318 urine specimens were inoculated directly into the medium suitable for demonstrating urease activity of T-mycoplasmas. The results of these tests in relation to the findings of T-mycoplasmas by culture on solid medium are shown in Table II. A turbidity in the test medium was observed in 32 (10%) of the 318 specimens. These 32 tests were considered as nonconclusive. A colour change in the urease test medium indicating production of ammonia was obtained in all instances in which T-mycoplasmas were found on the solid medium. A colour change was also obtained in 6% of the cases in which no colonies of T-mycoplasmas were found on the agar plates. In the four instances in which a colour change in combination with turbidity was obtained, T-mycoplasmas were not demonstrated by cultures from the urine directly on agar plates.

The results of the urease colour tests on the specimens collected by SPA were in all but 11 instances in agreement with the results obtained on the voided specimens collected from the same individuals. In these 11 instances there was a colour change in the tubes inoculated with voided urine but not in the tubes inoculated with SPA urine. In seven of these cases T-mycoplasmas were cultured from voided urine on agar plates, but not from SPA urine.

CASE REPORTS

Case 1

A 26-year-old, single man. In 1967 he was treated with sulfonamides for acute epidydimitis and orchitis. Cultures for gonococci and *Mycobacterium tuberculosis* had been negative. Bacteriuria had not been present. A routine examination in 1968 revealed pyuria and microscopic haematuria. BP 140/80 mmHg, Hb 14.8 g 100 ml, ESR 17 mm, WBC 2 700 mm³ serum creatinine 0.95 mg 100 ml. Maximal osmolality in urine after i.m. administration of 5 IU of pitressin tannate was 840 mosmol/kg. Intravenous pyelography showed a small right kidney (9 4 cm) and destruction of the papillae of the left kidney. Voiding urethrocystography was normal.

Over a 13-month period bacterial cultures were made from voided urine specimens on 11 occasions and twice from urine collected by SPA. Except for sparse growth of *Staphylococcus epidermidis* and diphtheroid rods in some of the voided specimens, the bacterial cultures yielded no growth. Cultures for *Mycobacterium tuberculosis* from three specimens were negative. Cultures for *Mycoplasma* were made from nine voided urine specimens. In four of the first collected samples there was heavy growth of T-mycoplasmas. In three of them there was also sparse growth of *M. hominis*. Culture from SPA urine also yielded heavy growth of T-mycoplasmas. Treatment with tetracycline was then instituted. No growth of *Mycoplasma* was obtained in the three voided urine specimens collected during a period of 5 months after the treatment.

Case 2

A 52-year-old female with proteinuria during the last trimester of her pregnancies in 1937, 1940 and 1953. Hypertonia and anaemia were found in 1963. In 1967 she had a serum creatinine of 0.9 mg/100 ml and a creatinine clearance of 94 ml/min. Urine sediments were normal and there was no proteinuria. Renal angiography showed double renal pelvices and ureters on the right side. The right and the left kidney measured 16 5.5 cm and 10.5 5.5 cm, respectively. Arterial changes and reduced cortex in the cranial pool of the left kidney were suggestive of pyelonephritis. A selective renal angiography 1 year later showed progress of the changes in the left kidney and at this time pyelonephritic lesions were found

also in the right kidney Voiding urethrocystography showed no vesico-ureteric reflux.

Bacterial cultures from voided urine specimens performed on 31 occasions from 1968 to 1970 yielded no growth of urinary pathogens. In some specimens α-streptococci and γ-streptococci were found. Cultures for tubercular bacilli were negative. *M hominis* was found in four of the voided urine specimens collected during the spring of 1968 The patient was treated with tetracycline, and subsequent cultures yielded no growth of *Mycoplasma* during that year Cultures from voided urine early in 1969 showed growth of T-mycoplasmas. In March 1969 urine collected by SPA yielded sparse growth of T-mycoplasmas, as did the voided urine specimen collected on this occasion. T-mycoplasmas were cultured from voided urine in two other specimens collected in the autumn f 1969 and from the single specimen obtained in 1970.

Case 3

A 33-year-old female. IV-para. In 1945 she was treated for acute pyelonephritis. Her first child died in 1961 t 1 month of age. A healthy child was born in 1964 During her last two pregnancies in 1968 and 1970 she had preeclampsia. Bacterial urine cultures showed no growth. In 1970 pyuria and persistent proteinuria (1 6‰) were detected. BP 140/80 mmHg, ESR 10 mm, Hb 14.6 g/ml, serum creatinine 1.2 to 1.3 mg/100 ml and creatinine clearance 80 ml/min. Maximal urine osmolality after i.m. administration of 5 IU of pitressin tannate was 690 mosmol/kg. Intravenous pyelography revealed small right kidney (9.5 4 5 cm) The renal cortex was reduced bilaterally The renal pelvis was enlarged on both sides, and both the ureters were dilated. Voiding urethrocystography showed vesico-ureteric reflux on the right side

On five occasions during 1970 bacterial cultures showed no growth. On one of these occasions urine was collected also by SPA. The SPA specimen and the voided urine specimen collected on the same occasion yielded heavy growth f T-mycoplasmas and sparse growth f *M hominis* without concomitant bacteria. After treatment with tetracycline there was no growth of *Mycoplasma* from voided urine specimens

DISCUSSION

The present study shows that *Mycoplasma* can be isolated from the urinary tract above the urethra. The role played by these organisms in infectious conditions of the upper urinary tract cannot yet be stated. The theoretical possibility that mycoplasmas may be recovered from bladder urine in healthy individuals may also be considered.

The technique of collecting urine for cultures by SPA provides an accurate method of diagnosing infections of the urinary tract above the urethra (1 6) The quantitative approach for the detection of bacterial infections within the upper urinary tract, by considering reproducible findings of more than 100 000 organisms per ml of voided urine as an indication of true bacteriuria (4), does not seem applicable with *Mycoplasma* because of the longer generation time of mycoplasmas than of established bacterial pathogens of the urinary tract. The collection of urine specimens by SPA seems to be a suitable method for determining whether mycoplasmas may occur in the urinary tract above the urethra.

In the present series T-mycoplasmas were recovered from bladder urine in 10 of the 18 cases (56%) in which these organisms were cultured from voided urine, or in 9 6% of all the 87 investigated cases. *M hominis* was cultured from SPA urine in 2 of 9 patients in whose voided urine specimens this organism was found.

M hominis is frequently found in the urethra and the cervix of women with infections of the lower genital tract, but comparatively seldom from gynaecologically healthy women (7). It is also known that women with urinary tract infections often have an attendant infection in the lower genital tract (5) In the present series only few women were subjected to a gynaecological examination, and thus the frequency of genital infections was not established. In 7 cases *M. hominis* was isolated from voided urine only The 2 cases in which *M hominis* was isolated from SPA urine both had chronic pyelonephritis. In one of them *M hominis* was found without concomitant growth of bacteria. This case (case 3) is described in detail above It may be concluded that cases with growth of *M hominis* from voided urine samples should be further examined for infections of the genital as well as the urinary tract.

It may be mentioned that in an earlier study of 40 women with acute salpingitis, *M hominis* was isolated from urethral specimens in 25 women and from urine collected by catheterization in 12 of these (9). A contamination of catheter urine specimens by mycoplasmas from the urethra can never be excluded, however The present study indicates that an examination for *Mycoplasma* in urine should include cultures from urine collected by SPA.

In contrast to *M hominis* T mycoplasmas have been recovered from the urethra and the cervix in about the same frequency in women with genital infections as in women in whom the gynaecological examination revealed normal conditions

(8). T-mycoplasmas have been isolated from the lower genito-urinary tract in about half of all women of childbearing age but only in isolated cases from healthy prepubertal girls or postmenopausal women. The organisms have been demonstrated about 30% more often from specimens collected from the urethra than from voided urine specimens of the same cases (8). It is therefore possible that the cases studied in the present series were harbouring T-mycoplasmas within the urethra more frequently than the figures of the isolation rate indicate.

T-mycoplasmas were never recovered on solid medium unless there was a colour change in the urease colour test medium, indicating ammonia production. A corresponding result was recently reported by Shepard and Lunceford in a study of the occurrence of T-mycoplasmas in urethral and voided urine specimens (11). In the present series a colour change was, however, observed in some instances (6%) in which no colonies of T-mycoplasmas could be found on the agar plates. This discrepancy might have been due to a failure in the culture of T-mycoplasmas on solid medium because of few organisms in the actual specimens, or as proposed by Taylor Robinson et al. (12) because epithelial cells in voided urine specimens may contain a urease. A contamination of the urease colour test tubes with urea-splitting micro-organisms is generally ruled out by a lack of turbidity of the test medium (11). A pure culture of T-mycoplasmas does not produce turbidity.

When using pure cultures of T-mycoplasmas, the urease colour test has an extremely high reliability. Our study indicates that the urease colour test can be used in clinical practice to exclude urine specimens not containing T-mycoplasmas. The sensitivity of the urease colour test in clinical use, e.g. its ability to select urine specimens containing T-mycoplasmas, and the specificity of the test, e.g. its ability to disclose findings of T-mycoplasmas, were found to be very high.

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HEPARIN RELEASED BLOOD PLASMA LIPOPROTEIN LIPASE ACTIVITY IN PATIENTS WITH HYPERLIPOPROTEINEMIA

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Abstract. Plasma lipoprotein lipase (LPL) activity appearance in the blood stream after different doses of 1 injection of heparin has been studied. After 100 IU of heparin/kg b.wt. LPL activity was found to rise rapidly for about 5 min and then more slowly up to maximum activity at 20 to 40 min. The possibility that the different appearance rates might reflect several enzyme pools not equally accessible to injected heparin has been discussed. Against this background plasma LPL activity as determined both at 5 and 40 min after 1 injection of 100 IU of heparin/kg b. w. in subjects w th wide range of plasma triglyceride (TG) concentrations. There was no relationship either in men or women between heparin-released plasma LPL activities and age or weight/height index. In men with normal plasma TG values on the day of heparin injection the heparin-released LPL activities in plasma were 49±2 and 100±4 μmoles glycerol released per litre plasma and minute in the early and late samples respectively. The corresponding values of 40±3 and 72 6 for men with hypertriglyceridemia were significantly lower. In women with normal plasma TG values the LPL activities 5 and 40 min after heparin were 71+4 and 127±7 μmol/l and min respectively. These values are significantly higher than the corresponding values for normal men. The women with hypertriglyceridemia had plasma LPL activities 5 and 40 min after heparin of 45±7 and 85±10 μmol/l and min, which are significantly lower than the corresponding values for the women with normal plasma TG concentrations. The significantly lower values of heparin released LPL activity found in patients with hypertriglyceridemia may reflect removal (clearing) defect of plasma TG which may provide an explanation of the elevated plasma TG levels in these patients.

There has been considerable interest in lipoprotein lipase (LPL) clearing factor lipase, activity since the report of Hahn in 1943 (11) as recent reviews reveal (12, 20). A central role for the enzyme system, LPL, in the removal of blood plasma triglycerides (TG) has been assumed. The determination of heparin-released LPL activity sometimes called postheparin LPL activity (PHLA) is today a clinical test for classification of patients with hyperlipoproteinemia (10). The only clinical condition at present clearly characterized by absence or low values of heparin-released LPL activity is fat-induced hyperlipaemia with a type I lipoprotein pattern. Many studies have been carried out to see whether plasma LPL activity after heparin is impaired in patients with ischaemic heart disease and/or hypertriglyceridaemia other than fat-induced hyperlipaemia. This subject has been thoroughly discussed in two reviews by Robinson (19, 20). There has been no agreement among the studies, some showing decreased values and others increased or unchanged values compared to controls. The reason for the disagreement might be due partly to methodological problems. In several of these studies the lipolytic activity has not been well characterized as LPL activity. Furthermore low heparin doses have often been used and only one sample has been analysed without knowledge of the time course of the appearance and disappearance of the LPL activity after the heparin injection.

A method of good reproducibility for the determination of heparin-released LPL activity using Intralipid® as substrate, has been described previously (2). Results of its use in the routine investigation of patients with hyperlipoproteinemia will be described in this report.

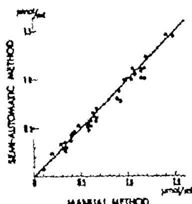


Fig. 1. Comparison of glycerol concentration of the incubation medium determined by new semi-automatic method and the former manual method in 40 samples. The line represents the equation $y = x$. The coefficient of correlation is 0.98 and the equation of the regression line is $y = x(0.94 \pm 0.03) + 0.01$.

MATERIAL AND METHODS

Control subjects. Eight male students, 20–.5 years of age, 15 male taxidrivers, 49–65 years of age, and 16 female telephone operators, 50–64 years of age, volunteered to take part in the study. They were all judged healthy from clinical history and from analyses of Hb levels of plasma transaminases, urinalysis of protein and glucose, and fasting values of plasma TG and cholesterol and of serum lipoproteins.

Patients. Males and females, 14–73 and 28–73 years of age respectively who on any occasion were found to have fasting plasma TG concentration above 2 mmol/l or a fasting plasma cholesterol concentration above 300 mg/100 ml were studied. They were patients from either the Department of Internal Medicine at the Karolinska Hospital or the Ersta Hospital, Stockholm. All patients with overt diabetes mellitus, as judged by the presence of glucosuria and fasting blood sugar level above 110 mg/100 ml, or with other endocrine disorders, as judged by history and physical signs, have been excluded.

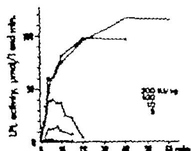


Fig. 2. Appearance of LPL activity in blood plasma after different i.v. doses of heparin. The heparin was given at zero time. Each symbol is the mean value from studies in 4 young healthy men.

General procedure. All subjects reported at the hospital between 8 and 10 a.m., having fasted overnight. No dietary modifications were made on account of the study. A polyethylene catheter was inserted percutaneously into an antecubital vein. The catheter was kept patent throughout the study by flushing it with saline. Before heparin was injected, blood sample was taken for the determination of TG (14), cholesterol (25) and, in all controls and about half of the patients, for lipoprotein analysis by ultracentrifugation (8) and paper electrophoresis (10). At zero time 100 IU of heparin (Vitrum, Stockholm, Sweden) per kg b.wt. was given i.v. Nine ml of blood was taken for LPL activity analysis 5 and 40 min after the heparin injection and transferred into glass tubes containing 1 ml of 0.1 molar trisodium citrate. The blood samples were kept on ice and then centrifuged at 4°C. After centrifugation plasma was frozen and LPL activity determined within two months (2).

Determination of LPL activity was carried out, with slight modification, as described previously (2).

The substrate mixture contained lyophilized human blood serum albumin (the same batch for all determinations), glycerol-free soybean oil emulsion (Intralipid minus glycerol, Vitrum), and ammonium buffer in the amounts described before (2). The incubation medium consisted of three volumes of substrate mixture and one volume postheparin plasma. The incubation conditions, pH and temperature were also as described before (2, 3). Glycerol concentration of the incubation medium was measured 30, 40, 50 and 60 min after the start of incubation, except for very low plasma LPL activities when the time intervals were doubled. Glycerol was determined by a semiautomatic method using Technicon Auto Analyser. Aliquots, duplicate 0.3 ml, of the incubation medium were transferred to glass tubes containing 3 ml of isopropanol. After vigorous shaking the protein precipitate was centrifuged to the bottom. Supernatant was decanted into Auto Analyser sample cups for the determination of glycerol (14). LPL activity is expressed as release of glycerol per unit time during the linear part of the glycerol release.

Appearance of LPL activity in the blood stream after five different i.v. doses of heparin (1, 5, 10, 100 and 200 IU/kg b.wt.) was studied in each of four men. For one subject only one dose was given per day and before the next study at least three days passed. For the lowest heparin dose (1 IU/kg) arterial blood and for the other heparin doses venous blood was sampled at 2, 3, 4, 5, 6, 8, 10, 12 and 15 min after the heparin injection. For the heparin doses of 5, 10, 100 and 200 IU/kg additional samples were taken at 20, 40 and 60 min after the injection. LPL activity in the samples was determined as described above.

RESULTS

Determination of heparin-released LPL activity. The new semi-automatic method for glycerol determination gave results identical with the former manual one (2) (Fig. 1). Regression ana-

Table I. *Range for age weight/height index, plasma TG and cholesterol concentration and mean* ± *S.E.M for heparin-released plasma LPL activity in controls and patients with hyperlipoproteinemia*

	Age (y.)	Weight/ height index	Plasma cholesterol (mg/100 ml)	Plasma TG (mmol/l)	LPL activity after heparin (µmol/l and min) 5 min	40 min	40 – 5 min
Men							
Young controls (n = 8)	20–25	0.76–0.96	135–175	0.73–1.67	48 ± 6	119 ± 10	71 ± 11
Old controls (= 15)	49–65	0 85–1.47	158– 40	0.98–1.70	51 ± 3	95 ± 5	44 ± 5
Patients (= 63)	14-73	0.73-1.57	150–460	1.05–67.90	43 ± 2	83 ± 4	38 ± 3
Women							
Controls (16)	50–64	0 84–1.43	185–281	0.90-2.25	69 ± 5	118 ± 7	49 ± 6
Patients (n 25)	28-73	0.81 1.58	188–900	0 91 54 40	57 ± 5	109 ± 9	52 ± 6

lysis resulted in the equation $y = x(0.94 \pm 0.03) + 0.01$, where y and x are glycerol concentrations determined by the new and old method respectively. This is not significantly different from the identity relationship $y = x$.

Appearance of LPL activity after intravenous injection of heparin. Fig. 2 shows that for increasing doses of i.v. heparin up to 100 IU/kg there is a progressive increase in plasma LPL activity. The LPL activity after 200 IU/kg was not significantly higher than after 100 IU/kg. The appearance curve of LPL activity after 100 IU/kg of heparin can be divided into two main parts, the first a very rapid appearance rate between 0 and about 6 min and thereafter a second slower appearance rate.

Thus, in clinical tests for heparin-released LPL activity the general procedure has been to use the heparin dose 100 IU/kg b.wt. and to take samples 5 and 40 min after the heparin injection. The LPL activity 5 min after the heparin injection is taken to indicate net increase in the blood during the rapid appearance phase, and the activity 40 min after should represent the peak or maximum value. The LPL activity 5 min after the heparin injection was, for the whole population studied, significantly correlated to the 40 min value ($r = 0.76$). The relationship was not significantly altered by sex, age or plasma TG concentration. There was no correlation between the LPL activity of the 5 min sample and the activity of the 40 min sample minus the 5 min sample ($r = 0.08$).

LPL activity and plasma TG concentration. Results obtained for the control groups and for the patients are presented in Table I. There was a

Table II. *The coefficients of correlation for the relationships between heparin-released plasma LPL activity and age, weight/height index and the log values of plasma TG levels in all subjects studied (86 men and 41 women)*

ns not significant, p statistical significance of the correlation coefficient

Plasma sample	Age (y.) Men	Women	Weight/height index Men	Women	Log. plasma TG Men	Women
5 min	0.04 ns	−0.21 ns	0.03 ns	0.16 ns	−0.20 ns	−0.53 $p < 0.001$
40 min	−0.02 ns	−0.03 ns	−0.11 ns	0.24 ns	−0.47 $p < 0.001$	−0.55 $p < 0.001$
40 5 min	−0.05 ns	0.01 ns	−0.15 ns	0.14 ns	−0.45 $p < 0.001$	−0.22 ns

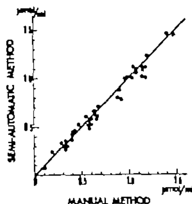


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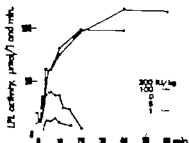


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RESULTS

Determination of heparin-released LPL activity. The new semi-automatic method for glycerol determination gave results identical with the former manual one (2) (Fig. 1). Regression ana-

Table III. *Plasma LPL activities (μmol/l and min) after intravenously given heparin (100 IU/kg b. wt.) in men and women with normal and pathologically elevated plasma TG concentrations*

Upper limit for normal plasma TG concentration was the population mean plus two standard deviations (log. values) considering age and sex.

	5 min after	40 min after	40 – 5 min after
Men			
Normals	49±2 (n=47)	100±4 (n=51)	50±3 (n=47)
Hypertriglyceridemia	40±3 (n=35) $p<0.02$	72±6 (n=35) $p<0.001$	32±4 (n=35) $p<0.001$
Women			
Normals	71±4 (=26)	127±7 (=77)	57±5 (26)
Hypertriglyceridemia	45±7 (n=14) $p<0.001$	85±10 (n=14) $p<0.001$	40±6 (=14) ns

plasma TG levels. Net increase of plasma LPL activity between 5 and 40 min after the heparin injection was significantly lower in men but not in women.

DISCUSSION

The present study has demonstrated a clinical application of a method for heparin-released plasma LPL activity Some years' experience has shown that the method is easy and rapid to per form and that the specificity and reproducibility are good when the conditions are standardized.

There is a rapid appearance of plasma LPL activity after i.v injection of heparin during the first minutes. At higher heparin doses a second, slower appearance is recognized and a maximum plasma level of LPL activity is reached. Net increase of the activity from 0 to 5 min after heparin injection was 60 μmol/l and min, and for the intervals 5 to 10, 10 to 15 and 15 to 20 min the net increase was between 10 and 20 μmol/l and min. This change in course of appearance might be explained by different enzyme pools. One pool is very accessible to the injected heparin, as reflected by initial rapid appearance. This lipoprotein lipase pool could be located t the luminal surface of the capillary endothelium. It was shown already in 1959 by Robinson and Harris (21) for the hind limb of the rabbit that, when Evans Blue dye was injected with heparin intra-arterially the dye and the lipolytic activity appeared in the same sample of venous blood" about 20 sec after the injection. The second part of the appearance curve could then reflect an other less accessible pool located extravascularly (22) It has been postulated that lipoprotein lipase in rat adipose tissue is synthesized in the fat cell and is then transported out of the cell to a final location at the luminar surface of capillary endothelium. A similar origin has been suggested for heart muscle LPL activity (6, 7 2.) On the basis of this hypothesis and the time curve two samples were taken for the determination of heparin-released LPL activity The sample 5 min after the heparin injection would correspond to the easily accessible pool and the second sample taken 40 min after the injection would reflect the total heparin-releasable LPL activity The results showed that these two activities were fairly well correlated to each other This might very well be due to the fact that the 5 min alue is included in the 40 min value. However the net increase of plasma LPL activity between 5 and 40 min after the heparin injection (i.e. LPL activity of the 40 min sample minus that of the 5 min sample) was not correlated to the activity of the 5 min sample.

The easily accessible LPL activity measured 5 min after heparin, was significantly lower in patients with hypertriglyceridemia compared to the subjects with normal plasma TG concentrations. The LPL activity considered as extra vascular the 40 minus the 5 min alue, was lower in hypertriglyceridemia patients than in subjects with normal plasma TG but significantly so only for men.

One factor that makes it difficult to draw these conclusions from heparin released LPL activity in plasma of intact man is the uneven distribution of the activity between diff rent tissues. W know from studies in animals that there is a reciprocal relationship between LPL activity in heart muscle and in adipose tissue with changes in nutritional state (20) It has also been shown that LPL ac tivity in human adipose tissue decreases during fasting (18) Furthermore, large ariations in re gional production of heparin-released plasma LPL activity have been described (16) After injection of heparin in vivo available LPL activity

Table I. *Clinical data*

Case	Age (y)	Primary thyroid disease	Operation	Serum Ca limits before the test period (mEq/l)
1	73	Non-malignant toxic goitre	Subtotal thyroidectomy	3 96–2.86
2	55	Non-malignant toxic goitre	Subtotal thyroidectomy	3 17 2.54
3	49	Non-malignant toxic goitre	Subtotal thyroidectomy	4 79–4.05
4	45	Carcinoma	Total thyroidectomy	7.23–4.57

Spectrophotometer 153, coefficients of variation 1 4% and 0 respectively). Sodium and potassium were measured by emission flame photometry (coefficients of variation 1 and 1 8% respectively), creatinine with Technicon Autoanalyzer (coefficient of variation 3 4), and phosphate by modification of the method of Fiske and Subbarow (9) (coefficient of variation ~5%).

RESULTS

There was a fall in the serum calcium concentration in two patients. The other parameters measured in the blood were not affected by the calcitonin injection (Fig. 1) The test was interrupted in case 2 because of increasing paraesthesia, which disappeared immediately after an i.v. injection of a calcium salt. No side-effects occurred in the other patients.

There was a general tendency to an increased excretion of all electrolytes measured (Figs. 2-4) The most prominent changes took place in the excretion of sodium, which went up by 350-700%

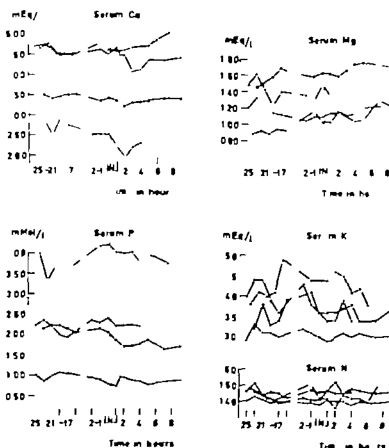


Fig. 1. Changes in the blood following the injection of calcitonin. —●— case 1 —○— case 2, case 3 – case 4

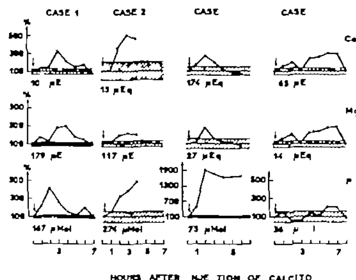


Fig. 2. The hourly excretions of Ca, Mg and P following the injection of calcitonin. The average hourly excretions of the control periods are indicated by the numbers underneath the baselines and represent the 100% values. (Hatched areas = mean ± S.D.)

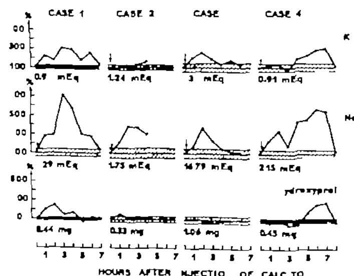


Fig. 3. The hourly excretions of K, N and hydroxyproline following the injection of calcitonin. The baselines are indicated as in Fig. 2.

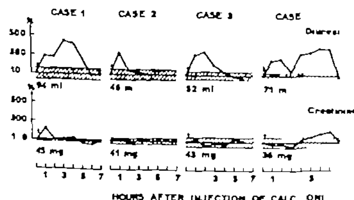


Fig. 4. The hourly diureses and excretions of creatinine following the injection of calcitonin. The baselines are indicated as in Fig. 2.

in the four patients. A marked phosphaturia was seen in cases 1–3 but not in case 4 The excretion of hydroxyproline was low in all patients, which was to be expected in this type of patient (3) No further reduction took place after the calcitonin injection.

The strong natriuretic effect of calcitonin was followed by increased diuresis in all patients (Fig. 4) We found considerable variations in the hourly excretions of creatinine, which is in agreement with the findings of several other investigators (14 22) but no particular changes in connection with the calcitonin injection.

DISCUSSION

The results indicate that calcitonin has an action on the kidney since the urinary changes in these four patients could not be explained by a secondary stimulation of parathyroid hormone Ardaillou et al. (1) found a significant increase of the renal excretion of calcium and phosphate in two hypoparathyroid patients given calcitonin. Singer et al. (19) obtained similar results in an other two hypoparathyroid patients and in addition, an increase of the excretion of magnesium and sodium.

The urinary changes might be due to impurities in the calcitonin preparations. The only study in man, however with synthetic human calcitonin comprises one hypoparathyroid subject, in whom the injection of 1.5 mg of the pure hormone also resulted in a significant increase in the phosphate excretion (8)

Calcitonin injections into rats induce a reduced renal excretion of calcium (15 16) In these animals the hormone produces very rapidly a decrease in the serum calcium levels with a concomitant decrease in the filtered loads in the kidneys. This obscures the hormonal effect upon the kidney Bone turnover in man is slow as compared to the rat, and the urinary changes will therefore occur before any changes take place in the blood.

The impressive natriuretic and diuretic effect of calcitonin is noteworthy Similar results have been obtained in rats (21)

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ANTIBODIES TO THE EPSTEIN BARR VIRUS IN KIDNEY TRANSPLANT RECIPIENTS

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Abstract. Changes in antibody titer against the Epstein-Barr virus (EBV), the apparent cause of infectious mononucleosis, have been surveyed in 30 patients, randomly selected from kidney transplant recipients in Aarhus during the last five years. Sera taken at transplantation and at approximately 1, 2, 3 and 5 to 12 months post-transplant were examined for antibodies against EBV using an indirect immunofluorescence technique with EB-3 cells. Anti-EBV antibody and thereby evidence of past infection, was found in all but one set of sera. In three patients significant, fourfold, rise in antibody titer was observed. One, a man of 31, had transient illness with fever, sore throat and enlarged lymph nodes in association with antibody changes; another, girl of 18, had two short febrile periods of unexplained etiology the third, girl of 11, had no recorded symptoms.

We have previously studied the incidence and clinical manifestations of infection caused by three of the human herpesviruses—herpes simplex, varicella-zoster and cytomegalovirus—among kidney transplant recipients in the Aarhus series (1 14) In the present report the situation concerning another member of the herpes group, the Epstein-Barr virus (EBV) the apparent cause of infectious mononucleosis, has been surveyed during the first 5–12 post-transplant months in the same patients.

MATERIAL AND METHODS

Patients

The study group consisted of 30 randomly selected patients from among those transplanted during the period May 1966 to Aug. 1970. There were 17 women and 13 men with an average age of 31 years (range 11 58). Twenty-eight had received an immunosuppressive drug, azathioprine, and large doses of prednisone, especially during the first post-transplant month, as anti-rejection measures. Two patients with isografts received only azathioprine, to prevent recurrence of glomerulonephritis. Details of treatment have been reviewed in previous publications (1, 14) and will not be dealt with here.

Sera

The sera used had originally been obtained as part of the study of cytomegalovirus infections. Serum had been taken at the time of transplantation and thereafter at monthly intervals. The period of serologic surveillance in the patients studied varied from 120 to 357 days. In all patients serum sample taken at the time of transplantation was included. In ten patients four samples were available in the others five or more sera were tested. A negative control serum was obtained from child and positive serum from patient with infectious mononucleosis. All sera had been inactivated at 56 C for 30 min and then stored at 20 C for six months to five years.

Serology

An indirect immunofluorescence technique, essentially that of Henle and Henle (6) as modified by Marker (11), employing arginin-starved, acetone-fixed EB-3 cells (Statens Seruminstitut, Copenhagen), was used to determine EBV antibody titer. All sera from each patient were assayed simultaneously using twofold serial dilutions starting with 1 20. Negative and positive control sera were included and the same source of antigen was used throughout.

Staining reactions were read in dark field in Zeiss fluorescence microscope with HBO-200 lamp and interference filters especially adjusted to fluorescein isothiocyanate (13). Positive sera gave strong apple-green fluorescence in 2 to 5% of test cells. This was seen either as diffuse granular pattern or as solid area of light.

Heterophil antibodies were tested for after absorption with guinea-pig tissue and ox red blood cells as described by Buescher (2), and the titers given are those obtained after guinea-pig tissue absorption.

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A POPULATION STUDY ON MONOCLONAL GAMMAPATHY

Follow-up after 5½ Years on 64 Subjects Detected by Electrophoresis of 6 995 Sera

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Abstract. Serum electrophoresis included in medical survey in 1964 of 6 995 subjects above 25 years of age revealed M-components in 64. One had myelomatosis. Five and half years later 16 persons had died and 9 had moved or refused to take part in this study The concentrations of the M-components in the remaining 39 cases were essentially unchanged. This finding suggests benign, monoclonal gammapathy The series represented an observation time of 247 patient-years, during which malignant gammapathy had not developed. Among the 6 931 subjects without serum M-components tw had been hospitalized for myelomatosis during the 6 years after the mass examination. In one of these there was only urinary discrete component and in the other the diagnosis was not well founded.

In the spring of 1964 a medical survey of the population above 25 years of age in a district of Sweden was extended to include paper electrophoretic screening of 6 995 consecutive serum samples (1) to assess the prevalence of monoclonal gammapathy

M-components (γG γA and γM) were demonstrated in sera from 64 persons. In 10 cases the concentration was 1.0 g/100 ml or more and as highest 2.4 The state of health of the persons with serum M-component was fairly good for their ages. Myelomatoses was diagnosed in one case and lymphatic leukaemia in one. None showed the clinical picture of Waldenstrom s macroglobulinaemia.

Two and a half years later 52 persons available were reviewed (2). All of them still had M-components and in roughly the same concentrations as at the first examination.

In the autumn of 1969 5½ years after the first examination, 39 persons available were reviewed. We also made an inventory of patients with myelomatosis, admitted between June 1964 and May 1970 to hospitals in neighbouring districts, in an attempt to find out whether any of the 6 931 persons without serum M-components in 1964 had developed myelomatosis.

MATERIAL AND METHODS

Of the 64 subjects with serum M-components in 1964 3 had moved from the region, 6 refused to take part in the investigation and 16 had died

As the region of the investigation is about 650 km from Malmö, it was not possible to examine the subjects personally A nurse visited the 39 persons available. Sera ere sent to Malmö and examined by paper electrophoresis as described previously (1)

RESULTS

Data concerning the 16 subjects who had died since 1964 are given in Table I. In only one case autopsy had been performed, without histological examination of the skeleton.

Most of the 39 subjects who took part in the investigation were fairly healthy for their ages.

The result of the serum electrophoresis is given in Fig. 1 In none of the cases had the M-component disappeared. In case 7848, a 55-year-old healthy woman, the concentration of the M-component (γA) had decreased from 1 7 to 1 1 g/100 ml. Case 6140 was a 67 year-old man who in 1964 had easy bruisability and wasting of muscles (1). In 1964 the concentration of his M-component (γG) was 1.3 in 1966 1 7 and in 1969 2.0 g/100 ml. His state of health was unchanged. The largest increase of the M-component concentration was seen in case 6350. The patient

Table I *Data concerning the 16 subjects who had died since 1964*

Subject no.	Time of death (mo./y.)	Age at death (y.)	Cause of death
6497	9/64	75	Malignant tumour
7953	10/64	61	Complication of cholecystectomy
3396	2/65	84	"Senility"
5457	10/65	70	Pulmonary embolism after appendectomy
3561	6/66	71	Cerebrovascular lesion
7006	11/66	69	Myocardial infarction
8290	12/66	84	Myocardial and cerebral infarction
4412	12/66	70	Myocardial infarction
8469	1/67	70	Cerebrovascular lesion
3860	3/67	70	Pancreatic cancer
5062	7/67	66	Cerebrovascular lesion
6230	10/67	87	Prostatic cancer
4070	11/67	82	Cardiosclerosis
6844	1/68	77	Myelomatosis, broncho-pneumonia
6794	5/68	78	Cardiosclerosis
7913	6/69	67	Malignant tumour

was a 92 year-old man with lymphatic leukaemia and an M-component of type γG in concentrations of 1.3 1.7 and 2.2 g/100 ml in 1964 1966 and 1969 respectively He was in a good general condition

Between June 1964 and May 1970 116 patients were treated for myelomatosis in hospitals serving the district of the investigation. Two of these 116 patients had taken part in the health control in 1964 and at that time no serum M-component had been found. The histories of these two patients are given below

CASE REPORTS

Case 73 4

Female born in 1922. Cholecystectomy was performed in 1963 In April 1968 thoracic pain developed and in August osteolytic lesions were detected in the ribs, pelvis and the 11th thoracic vertebra. A bone marrow smear contained 50% plasma cells No serum M-component was found but a light-chain component was demonstrated in the urine Despite treatment with X-ray melphalan and later also cyclophosphamide the disease progressed and at the end of 1969 she died with multiple, osteolytic lesions and hypercalcaemia. The serum sample from 1964 was no longer available but judging from a comparison of the electrophoretic strip from 1964 with strips from subjects examined at the same time the γ-globulin concentration was normal.

Case 7591

Female born in 1905 Because of diffuse pain in arms and legs she sought medical advice in March 1959 Physical examination revealed nothing abnormal except hypertension (240/110 mmHg). ESR 7 mm/1 h, Hb 14.4 g/100 ml, no albuminuria, nonprotein nitrogen 36 mg/100 ml ECG and cardiac X-ray revealed nothing remarkable. In October 1959 she was hospitalized because of a cerebrovascular lesion with right-sided paresis and was sent home in March 1964 In April 1964 she took part in the health survey control. She was admitted to hospital in January 1967 because of nausea and vomiting. Physical examination revealed nothing of interest except sequelae after the cerebrovascular lesion and auricular fibrillation. Hb 9.3 g/100 ml, RBC 3.4 mill., WBC 6 000. The bone marrow contained 6% plasma cells. ESR 54 mm/1 h. Serum electrophoresis: albumin 2.7 α_1 0.3, α_2 0.5 β 0.3 and γ-globulin 2.1 g/100 ml, part of which was an M-component. The immunological type of the M-component was not determined, and electrophoresis of the urine was not performed. Bence Jones heat test was negative. There was albuminuria + + + and visible haematuria. The urine contained leucocytes and bacteria. Serum creatinine 1.6 mg/100 ml. Skeletal X-ray revealed no osteolytic lesions. The diagnosis of myelomatosis was discussed, but because of her poor general condition cytostatic therapy was not offered and she was sent to a department of chronic diseases, where she died some months later Autopsy was not performed.

COMMENTS

The purpose of this follow up was to ascertain whether the monoclonal gammapathy found 5 years previously could still be regarded as benign and stationary A steady concentration of an M-



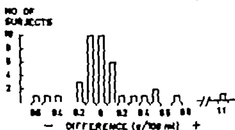


Fig 1 Concentration of M-component in the 39 subjects reviewed and differences in concentration of M-component between first and last examination.

component strongly suggests benign, monoclonal gammapathy (4 5) Of 39 components only two had increased more than 0.5 g/100 ml. The state of health of these two subjects was good. Excluding patient 6844 (myelomatosis) and the nine subjects who had moved from the area or refused to take part in the follow-up, the series represented an observation time of 247 patient-years without unequivocal development of malignant, monoclonal gammapathy

Considerable interest has been focused on the possible relationship betweeen malignant tumours and monoclonal gammapathy (3 4 6) It is true that the autopsy frequency in this series was low and that the survivors were not examined for malignant tumours other than malignant, monoclonal gammapathy This unselected series, however like the Malmö series, in which the autopsy frequency was high (4) lent no support to the assumption that malignant tumours, other than myelomatosis and Waldenström's macroglobulinaemia, can give rise to M-components. Nor except for diseases of old age, was there any clustering of any particular condition other than malignant tumours.

The rough method used to find out whether any of the 6 931 subjects without serum M-component in 1964 had developed myelomatosis within the following 5½ years revealed two cases. One of these two patients had a clear-cut myelomatosis but only a urinary light-chain component. Her electrophoretic strip from 1964 showed a normal concentration of gammaglobulin, which suggested that she had not had myelomatosis at that time. In the other case it was not possible to decide from available data, whether the M-component was a sign of myelomatosis.

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CENTRAL HAEMODYNAMIC EFFECTS OF DIHYDROERGOTAMINE IN PATIENTS WITH ORTHOSTATIC HYPOTENSION

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Abstract. The effects of dihydroergotamine (DHE) on *heart rate, cardiac output, stroke volume, arterial and* central venous pressures, systemic resistance, and central blood volume have been analysed in ten patients suffering from orthostatic hypotension. The patients were placed on tilt-table and studied in the supine as well as in the erect body position. In the supine position DHE caused significant increase of cardiac output, stroke volume, arterial systolic and mean pressures, and central venous pressure. In the erect position DHE raised cardiac output, stroke volume, and central blood volume, and lowered heart rate. Systemic resistance was unaltered by the drug. The effects of DHE on the central circulation can largely be explained by its powerful and relatively selective constrictor action on the capacitance vessels in the peripheral circulation. The results indicate that DHE can have beneficial effects in patients with orthostatic hypotension.

A shift of body posture from lying to quiet standing is associated with cardiovascular adjustments which tend to compensate for the effects of gravity on the circulatory system. The control of the capacitance vessels is of utmost importance in this situation in order to hinder excessive accumulation of blood in the dependent regions. The compensatory mechanisms seem to be insufficient in some subjects who show an orthostatic intolerance to standing in terms of an excessive increase in heart rate, a fall of systolic blood pressure, a decrease of pulse pressure and various subjective symptoms, such as palpitations and signs of relative cerebral ischaemia. This circulatory disorder can be seen in otherwise healthy people most commonly young women, and is often called orthostatic hypotension.

Subjects who displace an abnormal amount of blood into dependent regions during quiet standing might be relieved of their orthostatic symptoms by a vasoactive drug exerting a powerful constrictor action on the capacitance vessels, especially on those in the limb circuits. In a recent study (4) it was shown that dihydroergotamine (DHE) can elicit such an effect: both in normal humans and in orthostatic patients it evoked strong constrictions of the capacitance vessels in skeletal muscle and skin tissues, but had comparatively small or no constrictor effects on the resistance vessels and the precapillary sphincters; in fact, the resistance vessels of muscle might even show a slight dilatation. Animal experiments revealed that the effects of DHE on the intestinal and renal circuits were relatively insignificant. The results of that study indicated that, in the supine adult subject, DHE might cause a mobilization of about 350 ml of blood from the capacitance vessels of all skin and muscle regions and that, in the erect subject, the amount of blood pooled in dependent regions might decrease by some 125 ml after DHE administration. Such effects would markedly influence general cardiovascular haemodynamics and it was suggested that DHE might have beneficial effects in patients with orthostatic hypotension, an opinion supported by previous studies by Rosmanitz et al. (6) who observed a normalization of arterial pressure and heart rate in such patients after DHE treatment.

The aim of the present investigation was to analyse directly the action of DHE on central cardiovascular dynamics by determining its effects on cardiac output, stroke volume, central blood volume, and total systemic resistance. The investigation was performed on patients with orthostatic hypotension who were studied both in supine and erect body posture before and after DHE administration. Such a study does not seem

Table I. *Results of orthostatic test in patients selected for investigation*

	Heart rate	Systol. art. pressure	Diastol. art. pressure	Pulse pressure
Control data before test (mean ± S.D.)	85 ± 12.7 (beats/min)	125 ± 13.7 (mmHg)	73 ± 9.5 (mmHg)	53 ± 11.4 (mmHg)
Maximal change in during test (mean and range)	−43 (28 to 71)	−16 (−4 to −50)	+24 (+8 to +42)	−60 (−50 to −73)

to have been performed previously. Harris et al. (2) studied the central haemodynamic effects of DHE in patients during cardiac catheterization. Since that investigation, however, was performed only on supine subjects who, before the drug administration, seemed to deviate considerably from basal conditions (tachycardia), the results are not directly comparable to the present ones.

MATERIAL

The investigation was performed on subjects who fulfilled certain criteria (subjective and objective symptoms) of orthostatic hypotension that was not secondary to any other disease or induced by drugs. They were selected among a group of patients (mostly outpatients) who all suffered from several of the following subjective symptoms of orthostatic hypotension upon rising: palpitations, blurring of vision, dizziness, weakness with cold perspiration and [illegible] and syncope. T[illegible] be included in the material they [illegible] should exhibit [illegible] least two of the following objective signs of orthostatic hypotension at a routine orthostatic [illegible]: an increase of heart rate by more than 25 [illegible] [illegible] of systolic BP by more than 15 [illegible] change [illegible] increase) of diastolic pressure by more than 10% and decrease of pulse pressure by more than 40 [illegible] compared to the values at rest in supine position (cf. [illegible]). These values refer to the maximal changes of heart rate and of systolic BP and to the concomitant diastolic pressure observed during the orthostatic test, which implied quiet standing without support, usually for 8 min after [illegible] preceding period of rest. In this test heart [illegible] (ECG) and BP (auscultatory method) were measured [illegible] before and every minute during standing.

The study was performed on 10 patients who fulfilled these criteria. They [illegible] all [illegible], 17 to 64 years old (mean 28 years), 156 to 174 cm tall (mean 163 cm), and weighed 48 to 76 kg (mean [illegible] 7 kg). Eight of these patients fulfilled [illegible] least three of the criteria of the objective symptoms. The remaining [illegible] patients fulfilled formally only two of these, because they [illegible] about to faint, and diastolic pressure was not obtained at the maximal fall in systolic pressure. The mean results of the orthostatic test for the whole group are presented in Table I (diastolic and pulse pressure for only eight patients) as well as the control data at rest before the test.

METHODS AND EXPERIMENTAL PROCEDURES

The haemodynamic study on the selected patients was performed shortly (usually within 8 days) after the [illegible] orthostatic test and at a time when the subjects still suffered from orthostatic symptoms. These experiments started in the morning after the patients had had [illegible] light meal. A polythene catheter was inserted percutaneously via a cubital vein in the right arm and advanced to a site in the superior caval vein close to the right atrium. It was used for measurement of central venous pressure and for injections. Another catheter was introduced into the left brachial artery for measurement of BP and for sampling of blood during the determinations of cardiac output.

The BPs were measured by inductance manometers and recorded on a direct writing electrocardiograph (Elema [illegible]). The pressure transducers were placed at heart level, i.e. in supine body position 5 cm below the sternal angle, and in erect position at the sternal insertion of the fourth [illegible]. Mean arterial pressure was obtained intermittently by electrical integration. Heart rate was monitored from ECG recordings. Cardiac output was measured by the indicator dilution technique with bromsulphalein as indicator (9). Central blood volume was calculated as the product of mean transit time and cardiac output. Mean transit time was derived from the indicator dilution curve as Σ(concentration · time)/Σ(concentration). Total systemic resistance was calculated in resistance units, mmHg/(l/min), i.e. (mean arterial minus central venous pressure)/cardiac output.

The experimental procedures are shown in Fig. 1. After insertion of the catheters the patient was placed on a tilt table equipped with a saddle. With this support the patient was exposed to "passive standing" [illegible] tilted to upright position (85 to 90° angle from the horizontal plane). Heart rate and arterial and venous pressures were recorded continuously and cardiac output was measured at intervals in supine and erect position. The experiment started with a tilting procedure to acquaint the patient with the experimental steps and this included a simulated determination of cardiac output (injection of saline). It will be seen from the figure that, both before and after the administration of DHE, two determinations of cardiac output were made in the erect, and one in the supine position. In the erect position the determination was performed about 5 min after tilting.

Dihydroergotamine-methane-sulphonate, DHE (Ergonorm®, Dihydergot, Dihydroergotamine-Sandoz, Sandoz

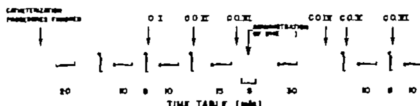


Fig. 1. The experimental procedures and timetable of the haemodynamic study in orthostatic patients. Cardiac output (C.O.) was determined in supine and erect (■) position before and after the administration of DHE.

was injected i.v. during a period of 5 min in a dose of 10 μg/kg b.wt. (In 2 cases 15 μg/kg). DHE was diluted in saline (1:10 v/v). Since the patients often experienced some discomfort for 10 to 20 min after the drug injection, the period of rest was extended to about 30 min before the next tilting. In general it was possible to follow the timetable of Fig. 1 but in some experiments slight deviations could not be avoided.

The total amount of blood withdrawn for the six determinations of cardiac output was about 60 ml.

In the statistical evaluation below p values less than 0.05 were considered significant.

RESULTS

The main purpose of the present investigation was to analyse the effects of DHE on central haemodynamics, especially on cardiac output, stroke volume, and central blood volume under relative steady state conditions in the supine and in the erect body positions. Since the circulatory state is less stable in the erect than in the supine position, it was considered desirable to obtain duplicate determinations during standing from two consecutive tilt manoeuvres both before and after DHE administration (Fig. 1) and to use the mean values of these two observations in the comparative analysis.

Information about possible beneficial effects of DHE in the orthostatic patient would be most readily obtained by comparing the circulatory data before and after drug administration in the supine position, on the one hand, and in the erect position on the other. Such a comparison is presented in Table II, which shows the mean results obtained in the ten patients in the two body positions as well as the calculated mean changes evoked by DHE. It also provides information about the haemodynamic changes elicited by the shift in body posture per se before and after DHE administration.

In the supine position cardiac output, stroke volume, arterial systolic and mean pressures, and central venous pressure were augmented significantly after DHE administration. The increase of cardiac output was caused by the increased stroke volume, since heart rate was not altered. The rise in arterial pressure, in turn, can be ascribed to the increased cardiac output, since total systemic resistance was not changed. DHE caused no significant increase of the central blood volume in the supine position.

In the erect position DHE caused a significant increase of cardiac output, stroke volume and central blood volume and a significant decrease of heart rate. The augmentation of cardiac output was thus related to the increased stroke volume. The systolic and mean arterial pressures rose after DHE administration in seven out of ten patients, but these increases were not significant. Total systemic resistance was not affected by DHE, nor did the drug raise central venous pressure in this position.

The patients experienced some untoward effects of DHE in terms of mild (in one case severe) nausea, slight dizziness, or a feeling of oppression in the chest or in the head. These side effects subsided within 10–20 min. In no case were any pathological changes in the ECG noted.

DISCUSSION

As mentioned, a previous detailed analysis of the effects of dihydroergotamine on the peripheral circulation in humans has shown that the drug exerts a fairly selective and powerful constrictor action on the capacitance vessels without much affecting the resistance function or capillary exchange function in the tissues (4). The consequent mobilization of blood from the peripheral blood depots (calculated at some 350 ml) in the supine subject, and decreased pooling of blood in dependent regions (calculated at some 125 ml) in the erect subject would be expected to markedly af

Table II. *Haemodynamic effects of DHE in supine and erect position*

	Heart rate (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	Arterial pressure (mmHg) Systolic	Diastolic	Mean	Central venous pressure (mmHg)
Supine							
Before DHE administration (mean ± S.D.)	67±8.7	5.2±0.69	79±7.8	120±7.8	69±7.5	88±6.1	5±3.4
After DHE administration (mean ± S.D.)	67±9.3	5.7±0.77	85±11.5	131±12.0	74±3.1	97±6.5	8±3.6
Mean change ± S.E.M.	+0.6±1.87	+0.43±0.157	+6.1±2.07	+11.4±2.15	+4.8±2.13	+9.3±1.66	2.7±0.7
Significance	N.S.	$p<0.025$	$p<0.02$	$p<0.001$	N.S.	$p<0.001$	p [illegible]
Erect							
Before DHE administration (mean ± S.D.)	89±9.4	4.3±0.53	48±5.4	119±10.8	72±6.2	90±8.0	2±4.8
After DHE administration (mean ± S.D.)	85±12.1	4.9±0.42	58±6.0	124±9.2	76±6.4	95±6.4	2±4.0
Mean change ± S.E.M.	4.0±1.76	+0.61±0.170	9.8±1.48	5.8±2.82	+3.1±2.17	+4.9±2.42	+0.2±0.99
Significance	$p<0.05$	$p<0.01$	$p<0.001$	N.S.	N.S.	N.S.	N.S.

fect general cardiovascular dynamics: DHE might tend to increase central venous pressure and central blood volume and thereby augment stroke volume and cardiac output which, in turn might raise systolic and mean arterial pressure without any concomitant major changes of systemic resistance.

The present study, in which the central cardiovascular adjustments evoked by DHE were analysed directly, confirms these conclusions in all essential respects. In supine subjects all these effects, except for an increased central blood volume, were present after DHE administration; in erect subjects all but the theoretically predicted pressure changes were revealed. The slight deviations from the expected pattern of response may be readily explained.

Before the administration of DHE central blood volume and central venous pressure were, as expected, greater in the supine (1.25 l and 5 mmHg, respectively) than in the erect position (0.88 l and 2 mmHg, respectively) (Table II). Owing to the consequent different pressure/volume relationship within the central circulatory system in the two body positions, a mobilization of blood from peripheral depots evoked by DHE might, in the supine subject, cause only slight additional accumulation of blood within the central circulation proper but nevertheless a clearly raised central venous pressure. In the erect subject, on the other hand, most of the mobilized blood can be deposited within the central circulation (some 120 ml, Table II) without leading necessarily to a measurable increase of central venous pressure. (A small change of central venous pressure is difficult to reveal in the standing subject due to possible minute involuntary changes in body erectness which will affect the pressure reference level.) The investigation indicates, however, that, in both body positions, DHE caused such adjustments within the central circulation that diastolic filling of the heart was improved, stroke volume was significantly increased (by 6 ml in supine and 10 ml in erect position) and so was cardiac output (by 0.43 and 0.61 l, respectively) (Table II).

It may be mentioned that the patients did not show the same pronounced orthostatic reaction during the haemodynamic study as during the preceding routine orthostatic test. Although, before DHE administration, systolic pressure fell

Total systemic resistance (units)	Central blood volume (l)	
16.0 ± 2.33	1.25 ± 0.133	10
16.0 ± 2.98	1.29 ± 0.170	10
0.05 ± 0.576 N.S.	+0.048 ± 0.033 N.S.	10
20.8 ± 2.67	0.88 ± 0.126	10
19.1 ± 1.84	1.00 ± 0.073	10
1.74 ± 0.801 N.S.	+0.120 ± 0.028 $p < 0.005$	10

fall more during the periods of tilting than indicated by the pressure value in Table II (119 mmHg) which refers to the moment just before the cardiac output determination, the patients no doubt seemed to have recovered to some extent from their previous orthostatic reaction. It is well-known that apprehension cannot be entirely avoided in subjects exposed to intravascular instrumentation, which would tend to increase the sympathetic vasoconstrictor fibre discharge, especially during the initial catheterization procedures. Increased sympathetic activity for one thing, causes a resetting of the pre- to post-capillary resistance ratio in the peripheral vascular circuits, so that mean hydrostatic capillary pressure falls and, thereby considerable amounts of extravascular fluid are absorbed into the circulatory system (3, 5). Such a reflex increase of plasma volume elicited during the period of catheterization in the studied subjects might very well have reduced their tendency to exhibit a pronounced orthostatic reaction during the haemodynamic investigation. In fact, a similar reflex increase of plasma volume, although less rapid, may be a normal compensatory event in orthostatic patients elicited during the daytime, when the sympathetic activity is known to be greater than when sleeping. A gradual increase of plasma volume during the day may explain the common experience that orthostatic symptoms are more pronounced in the morning than in the evening. Despite the possible partial normalization of the orthostatic reaction in the subjects of this investigation, it could be shown that DHE caused a clearcut increase of central blood volume, stroke volume and cardiac output in the erect body posture.

In conclusion it may be stated that DHE is able to improve the central haemodynamic state in orthostatic patients in a way that would be expected from a drug known to exert a powerful and relatively selective constrictor action on the peripheral capacitance vessels (4). The results from the present investigation and from that just mentioned, taken together with those of Rosmanitz et al. (6) who observed a normalization of blood pressure and heart rate in orthostatic patients after DHE treatment, strongly suggest that DHE can have beneficial effects in patients suffering from orthostatic hypotension.

ACKNOWLEDGEMENT

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DRUG-INDUCED AGRANULOCYTOSIS

II. *The Role of Medication in a Fatal Outcome*

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Abstract. Six fatal cases including material from 40 patients with agranulocytosis are described in detail, paying special attention to the drugs given before and after the onset of symptoms and recording the absence of neutrophils. Five of the six were cases of typical acute agranulocytosis and in four of them the drug probably causing the disease was still given after the recording of the low leukocyte count. The drugs given were aminophenazone, metamizol, sulfamethoxypyridazine and carbimazole. The authors conclude that agranulocytosis is rarely a fatal disease if the administration of dangerous drugs is stopped when the first clinical signs of agranulocytosis appear. Otherwise the administration of antibiotics and corticosteroids is not life-saving. Therefore information from patients and nurses about the first symptoms of agranulocytosis is very important when leukotoxic drugs are used.

We have earlier described a series of 63 occurrences of agranulocytosis in 51 patients during the years 1950–68 (8). In that paper we concluded that "idiopathic" agranulocytosis is a very rare disease if it exists at all. In our material only 11% of the cases were without any history of drug use, whereas in the other materials published the percentage of cases with negative drug history varies from 20 to 61 (1–5).

According to our earlier findings (8) the prognosis of agranulocytosis has improved remarkably since 1950. During the years 1950–54 the mortality rate was 83%; during 1965–68 it was only 13%. This is lower than the mortality rates we have found in the literature or other sources (2, 7, 9). Consequently, as we had accurate drug histories of nearly all our patients with agranulocytosis during the years 1960–68, we re-examined the case histories in order to find the reason why some of them proved fatal, paying special attention to the drugs given before and after the onset of symptoms and the disappearance of neutrophils, and to possible mistakes in medication.

MATERIAL

The material in its entirety has been described previously (8). For the subject of the present study we selected from the original material those cases which ended in death during the years 1960–68. We have not taken into account the corresponding cases before 1960 because of the inadequate histories taken before that time. Attention has been paid to the drug history before and after the clinical symptoms of agranulocytosis appeared and the diagnosis was confirmed, to the possible differences in the clinical and haematological picture of fatal cases compared with those of the surviving patients, and to the final cause of death.

The material consists of six patients. An autopsy was performed in each case.

CASE REPORTS

Six of 40 cases (15%) of agranulocytosis in our original material ended in death during the years 1960–68. Five of these six were women. The average age of the whole agranulocytosis material during the years 1960–68 was 49.7 years, of the fatal cases 48.5 years and of the surviving cases 48.1 years. These differences are not statistically significant.

The fatal cases, the onset of symptoms, the day when the low leukocyte count was recorded, and the drugs related to the disease, are illustrated in Fig. 1.

Case 1

No. 19 in the original material, a 28-year-old woman, who had had agranulocytosis after the use of aminophenazone (aminopyrine, USP) for toothache. After recovery she had been warned against analgesics, but 4 months later she again took tablets containing aminophenazone for toothache. She was admitted to hospital because of fever of 4

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after 3 days of fever swelling of the legs and poor general condition. In hospital deep vein thrombosis of the calf, heart failure, and complete absence of granulocytes in the bone marrow were observed. The number of leukocytes in the peripheral blood was 1 300 and the differential count showed 2% monocytes, 97% lymphocytes and 1% plasma cells. She was treated with prednisone, penicillin and warfarin. I addition 1 g metamizol was given twice and 0.3 g aminophenazone once during her stay in hospital to relieve pains. She died on her 6th day in hospital. At autopsy no cells of the granulocytic series were found in the bone marrow. Small petechiae in the endo- and pericardium and several ulcerations in the ileum and caecum were observed. A thrombotic mass in the calf vein and several emboli in both lungs were located.

Case 5

No. 46 in the original material, a 71-year-old woman who suffered from relapsing cholangitis which had developed after cholecystectomy and choledochojejunostomy. There fore continuous sulfamethoxypyridazine therapy was used. Because of fever of 3 days' duration she was admitted to hospital. In hospital the patient was very ill with high fever and lung infection. The total number of leukocytes was 600/mm³ there were no cells of the granulocytic series. Very few granulocytic cells were found even in the bone marrow. The sulfonamide was continued for 3 days in hospital, and she received 0.1 g aminophenazone three times as an antipyretic. She died on her 6th day in hospital. The autopsy revealed pneumonic process in both lungs.

Case 6

No. 48 in the original material, 63-year-old woman who had suffered from high blood pressure and heart failure for several years. Two months before the last admission to hospital, thyrotoxicosis was diagnosed and to treat this she received 300 mg carbimazole daily. She was re admitted for adjustment of the treatment for thyrotoxicosis. She had fever 3 days prior to admission, and in hospital one nodus of the thyroid gland, icteric skin and pharyngitis were observed. The total number of leukocytes was 600/mm³ with 2% basophilic, 4% monocytic and 94% lymphocytic leukocytes. In the bone marrow thrombo- and erythropoiesis are normal. Only few myeloblasts and promyelocytes and no mature granulocytes were found. The patient was treated with prednisolone, digoxin, furosemide and penicillin. She received carbimazole until agranulocytosis was recognized on the 2nd day in hospital. She died on her 8th day in hospital. The autopsy finding showed complete absence of granulocytic cells in the bone marrow.

DISCUSSION

In two of the six cases described nothing except the absence of granulocytes from the bone marrow was found at autopsy. In addition lung emboli were found in two cases and lung infection (pneumonia) in one. We may conclude that in five of the six cases agranulocytosis and its complications have been the cause of death. In one case (no. 2) the malignancy of the lymph node may have been the basic cause of death, although it seems improbable because the lymphoma was found in one lymph node and there were no other signs of generalization of this disease.

One of the cases (no. 3) does not resemble a typical case of acute agranulocytosis. The history of this case is more like the chronic form of the disease described by Begemann (1). This form of agranulocytosis is characterized by a poor tendency to recovery and the prognosis is worse than that of the acute form. It is perhaps a form of aplastic anaemia.

When comparing the clinical picture and haematological findings of the four remaining fatal cases with those of the surviving patients, no significant difference is noticeable between the groups. Not even the autopsy gives any clear-cut explanation why these particular patients died. In two of the six cases there was a thromboembolic complication, but in limited material like this it may be just a coincidence.

Observations on the medication after the symptoms of agranulocytosis appeared and after the low leukocyte count was recorded are more interesting. *Four of the six patients received drugs which are known to cause agranulocytosis even after the low leukocyte count had been recorded.* In one case aminophenazone and chlorpromazine, in one case aminophenazone and metamizol (dipyrone, USP), in one case aminophenazone and sulfamethoxypyridazine and in one case carbimazole. This corresponds well with the findings of 100% fatality when aminophenazone is continuously administered to patients with agranulocytosis (6). In the two remaining cases tetracycline (no 2) and tietylperazine (no. 3) which belongs to the phenothiazide group, were given. In addition, in case 2 the influence of malignant lymphoma on the f tal result cannot be xcluded. Case 3 had a more chronic course, more resembling a form of aplastic anaemia.

Thus, in at least four cases, the reason why the treatment failed was that le kotoxic drug was gi en even after the onset of symptoms. In some cases occasional analgetics had been administered by the n rses, but in two cases (nos. 5 and 6) the situation had been realized too late by the

doctors, although the blood cell counts had already been recorded.

The availability of sulfonamides and antibiotics has been said to greatly improve the prognosis of agranulocytosis (1 2, 10). However in our series (8) the mortality rate was still 83 % during the years 1950–54 when antibiotics were already used. The 33 % mortality rate in 1955–59 is similar to other reports from that time (3) and from our own pediatric series (4) Many cases from the years 1950–59 were not as carefully documented as the later cases, especially concerning drug history Thus we cannot exclude a possible administration of causative agents, even during the disease as the reason for the fatality in those cases. In the light of the present fatal cases we may conclude that the administration of antibiotics or corticosteroids in agranulocytosis is not life-saving, although they may be valuable.

Because we were able to point to a serious mistake in the treatment of nearly all the fatal cases of agranulocytosis, it seems likely that acute *agranulocytosis is rarely a fatal disease if properly* treated, and if it is not a symptom of some underlying fatal disease This is entirely opposed to the opinion presented earlier (1 2) In support of our opinion we wish to point out that in our material the frequency of fatal cases during 1960–68 was lower than that in any material presented earlier Furthermore in each case we were able to show the use of drugs known to cause agranulocytosis after the beginning of symptoms or after recording of the low leukocyte count.

As a conclusion we would maintain that, because stopping the administration of dangerous drugs is the most important factor in the fate of the patients with agranulocytosis, one should:

1) be aware of the possibility of agranulocytosis when the first clinical symptoms appear

2) *inform* the patient of the possibility and of the clinical symptoms of agranulocytosis when suspicious drugs are prescribed

3) restrict to the absolute minimum the giving of drugs to hospital patients with agranulocytosis. The nurses especially should be ordered not to give any extra symptomatic medication without consulting the doctor

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COMPOSITION OF PERICARDIAL FLUID IN CHOLESTEROL PERICARDITIS

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Abstract. T cases of cholesterol pericarditis have been described. The cholesterol content of the pericardial fluid was in one case 798 and in the other 138 mg/100 ml, but the triglyceride content was very low. Lipid electrophoresis of the pericardial fluid, although it differed from that of the serum, gave inconsistent results. Protein electrophoresis gave similar results in the serum and the pericardial fluid, and the electrolytes were similar in pericardial fluid and serum. Of the enzymes studied, only the lactate dehydrogenase activity (LDH) showed elevated values in the pericardial fluid, with preponderance of LDH_5 isoenzyme.

The justification for a diagnosis of cholesterol pericarditis is based on the elevated cholesterol content of the pericardial fluid, which commonly leads to the precipitation of cholesterol into crystals in the pericardial sac. Since the first published case (1) a total of 53 patients with this disease have been reported (7). The etiology of cholesterol pericarditis seems to be variable, and the basic mechanism of elevation of the cholester ol content in the pericardial fluid is unknown (2).

In the reported cases attention is usually focused only on the cholesterol content of the pericardial fluid. In the present cases the conventional laboratory analyses of pericardial fluid were compared with those of blood serum in two patients with chronic pericardial effusion having an ele vated cholesterol content.

CASE REPORTS

Case 1

A 39-year-old managing director, admitted to the hospital for severe dyspnea. Sixteen years earlier he had suffered from polyserositis of unknown etiology. Effusion had then been found in the abdominal cavity pleural spaces and pericardial sac. The hospital treatment had lasted for 3 months. Aspiration of pericardial fluid had been per formed. While discharged, the hydrothorax and ascites had disappeared but the heart contour had remained enlarged in chest X-rays. Since then he had been taking 0.25 mg digoxin daily and had been ell for 15 years. One year before the present admission he suffered from acute respiratory infection, after hich shortness of breath and and peripheral edema developed. The digitalis dosage had been increased and supplemented by diuretics, without beneficial effect. Because his condition as deteriorating the patient was hospitalized.

The patient was dyspneic at rest. There as no cyanosis. The jugular veins were distended in the sitting position. The liver was palpable 3 cm below the costal margin and the hepatojugular reflux was positive. There was no peripheral edema. On auscultation third heart sound was audible but there were no murmurs. Lifting of neither the left nor the right ventricle was palpable. ECG showed regular sinus rhythm, 77/min, with low voltage deflection.

The results of routine hematological and urine analyses were normal and the ESR was 23. The tests for LED were negative. Paul-Bunnel, toxoplasma and tularemia tests were negative. A biopsy specimen from the rectal mucosa did not reveal signs of amyloidosis. Serum total protein was 7.2 g/100 ml, with normal distribution in electrophoresis. Immunoelectrophoresis was normal and serum lipid electrophoresis showed moderate and sharp β-fraction and weak pre-β.

In the chest X-ray examination the heart was enlarged widely in all directions. The right cardiac border was so diffusely distended that exact calculation of the heart volume was not possible. Catheterization of the right side of the heart was done but the catheter stopped in the right atrium and, despite many attempts, did not go further. The mean pressure in the right atrium was 23 mmHg ($a = 26$, $= 19$ $= 24$, $y = 20$) The cardiac output was 5.5 l/min. In angiography both ventricles appeared to be small and surrounded by thick homogenous mass. In the echocardiogram the mass between the pericardium and the epicardium was interpreted to be more dense than the usual pericardial fluid.

Partial pericardiectomy was performed. The pericardial space contained 1.5 l of turbid fluid. The centrifugate of this fluid contained 355 erythrocytes and 7 leucocytes/mm^3 and cholesterol crystals were seen. Staining of

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Table I. Results of determinations in patients and controls (means ± S.E.M.)

Group	n	Age (y)	Body fat[a] (kg)	Fat cell Weight[a] (μg)	Fat cell No. (10^{10})	Fasting insulin (μU/ml)	Sum of insulin (μU/ml)	Fasting glucose (mg/100 ml)	Sum of glucose (mg/100 ml)
Men									
Control	49	55	16±1	0.59±0.04	3.2±0.3	9±1	296±38	65±3	435±21
Diabetic	13	59±2	25±2 (33±5)	0.57±0.05 (0.75±0.04)	4.4±0.8	11±3	114±13	162±14	1 340±101
p	—	—	<0.001 (<0.001)	n.s. (<0.01)	n.s.	n.s.	<0.001	<0.001	<0.001
Women									
Control	23	52	19±1	0.62±0.02	3.3±0.2	5±1	223±34	71±2	434±12
Diabetic	11	61±2	21±1 (29±3)	0.64±0.03 (0.84±0.03)	3.6±0.2	10±3	133±18	160±11	1 230±107
p	—	—	n.s. (<0.01)	n.s. (<0.001)	n.s.	n.s.	<0.05	<0.001	<0.001

[a] Figures within parentheses are values corrected for weight loss in connection with the onset of diabetes (see text).

Randomly selected middle-aged men ($n = 49$) and women ($n = 23$) served as controls. These materials have previously been analysed and reported (5).

A detailed history was taken from the diabetic patients concerning body weight changes in the year before diagnosis of their diabetes. Patients and controls reported to the laboratory in the morning after an overnight fast, having avoided exercise and smoking that morning. Glucose (19) and heparin plasma triglyceride (12), cholesterol (13) and insulin (16) were determined in fasting venous blood. Glucose, 100 g dissolved in 200 ml water, was then ingested and glucose and insulin were determined again after 30, 60, 90 and 120 min in repeated venepunctures. The subjects were sitting in a chair in the laboratory during the study, lying only when samples were drawn.

Body fat was estimated from anthropometric measurements found to correlate closely with body fat measured in similar populations of middle-aged men and women from the same region (5, 6) by isotope dilution methods (20, 21, 22). Fat cell size was determined (25) in the gluteal region in the men and in the thigh of the women. These two locations have been found to be representative of the largest subcutaneous adipose tissue regions in such populations (5). The number of fat cells was approximated by dividing the amount of body fat by fat cell weight.

RESULTS

Table I shows that the diabetic patients, by definition, were characterized by high fasting blood glucose and a decreased glucose tolerance. Insulin values were depressed, although most patients no doubt demonstrated remaining insulin activity in plasma. Plasma triglyceride values were higher in the diabetic patients. Body fat was elevated in diabetic men but not in diabetic women. Fat cell size and number did not differ in diabetic patients in comparison with controls.

It was found that most of the diabetic patients had experienced a decrease of body weight in the year before their diabetes was diagnosed. If it is assumed that this weight loss was due to a reduction of body fat, an estimate of body fat at the time when the weight decrease started could be calculated. Similarly a corresponding fat cell size at that time could be calculated, assuming a constant number of fat cells.

Table I also shows the results of these assumptions. This calculated body fat was significantly increased in relation to the controls. So was also fat cell size.

There were no significant correlations between fat cell size or number or body fat, on the one hand, and any of the metabolic variables on the other.

DISCUSSION

The comparison between diabetic patients and controls thus revealed that, as far as adipose tissue data are concerned, diabetic men were more obese. Surprisingly however diabetic women did not differ from control women. This could be explained by the fact that most of the patients had decreased in body weight in immediate association with the onset of their diabetes mellitus.

The empirically well-known loss of body weight associated with the onset of clinical diabetes

Triglyceride (mg/100 ml)	Cholesterol (mg/100 ml)
116±6	239±6
194±37	270±7
<0.05	n.s.
90±6	251±10
145±21	264±11
<0.01	n.s.

mellitus has different causes. Most important is probably that body fat decreases due to an insufficient inhibition of fatty acid release from the fat cells by insulin, as indicated by elevated plasma free fatty acid concentration in this condition (3). Also an increased free fatty acid release and lipolysis have been found in vitro in adipose tissue taken from patients with diabetes mellitus and obesity (9). A decrease in body cell mass also probably contributes to the body weight decrease due to increased gluconeogenesis with body proteins as substrate. The proportions between these contributions to weight loss are for obvious reasons not possible to examine retrospectively. The estimates of body fat before weight reduction are therefore only tentative.

The current concept of regulation of the amount of adipose tissue fat is that in adult age only fat cell size, but not fat cell number, is the factor that changes (17, 18). This, then, would mean that, when body fat decreased in the diabetic patients, this presumably affected only fat cell size while the fat cell number was constant.

It should be noted that fat cell size in the diabetic groups, when measured after weight decrease, was very close numerically to that of controls. With the assumption that body fat changes are expressed in adult age exclusively by changes in fat cell size, this then would very probably mean that fat cells were actually larger in the diabetic patients than in controls before the onset of diabetes, unless most of the weight loss was due to a decrease in lean body mass, which is highly unlikely. Although there were exceptions among the individual patients, this means that the well known body fat increase before onset of maturity onset diabetes is due primarily to an enlargement of fat cells or a hypertrophic type of obesity (10).

There was no statistical correlation between fat cell size and plasma insulin in these patients as found repeatedly in other populations (4, 6, 7, 8, 10). This is, however, not surprising in a condition where insulin secretion is severely impaired and the control of adipose tissue lipid mobilization, an important factor for the degree of filling of adipose tissue, is hampered.

It was recently reported that endogenous hypertriglyceridemia is also associated with increased fat cell size in adipose tissue (7). Endogenous hypertriglyceridemia and maturity onset diabetes mellitus may thus both be called hypertrophic obesity. The main difference between these two conditions is that endogenous hypertriglyceridemia has a glucose tolerance which is not decreased to such an extent as in maturity onset diabetes, presumably at least partly due to higher insulin secretion. The importance of insulin for elevation of plasma triglycerides in endogenous hypertriglyceridemia has been clearly demonstrated (14, 15, 23, 24). It is tempting to hypothesize that these two conditions differ mainly in regard to insulin secretion. When high, it is responsible for the maintenance of triglycerides in both adipose tissue and plasma in endogenous hypertriglyceridemia, while when clinical maturity onset diabetes mellitus is starting, triglyceride concentration in fat cells and in plasma can no longer be kept up due to insulin deficiency.

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LACTATE DEHYDROGENASE AND MECHANICAL TRAUMA OF ERYTHROCYTES

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Abstract. Intravascular hemolysis develops in most patients after prosthetic heart valve replacement. The serum lactate dehydrogenase activity (LDH) is elevated in these patients. It has been shown that the enzyme level correlates fairly well with the degree of red blood cell destruction. The heme pigments and LDH content of erythrocytes have been determined in ten normals and ten patients with prosthetic heart valves by hemolysing blood diluted in plasma. The red blood cells from the same subjects were exposed to standardized mechanical trauma by rotating the blood in cylindrical chamber. The LDH activity and heme pigment concentration were estimated in plasma before and after the rotation. The patients' red blood cells, subjected to the traumatic effect of the implanted valves, contained significantly less LDH than the normal erythrocytes. The in vitro experiments demonstrated that erythrocytes subjected to mechanical trauma lost proportionally much more of their LDH activity than of their heme pigments. This indicates that when the pigment loss is due to cell lysis alone, the LDH is derived also from erythrocytes remaining in circulation.

Replacement of diseased heart valves by ball valve prostheses provokes chronic intravascular hemolysis of varying degree in most cases (1 6, 7 10 11 12, 14). A rapid and fairly reliable method for evaluation of the degree of intravascular hemolysis is to determine the lactate dehydrogenase activity (LDH) in serum (9 10).

The relationship between serum LDH and intravascular hemolysis in these patients led us to study the effect of mechanical trauma on the LDH content of erythrocytes. In order to subject the blood cells to a homogenous trauma, the hemoresistometer of Fleisch and Fleisch was used, in which heparinized blood is rotated in a cylindrical chamber by a cubical rotor (3 4).

The aim of the present investigation was to estimate the LDH content of the erythrocytes in normals and in patients with prosthetic heart valves. Further we wanted to study the relationship between the loss of LDH and heme pigments from the red cells after a mechanical trauma in the same subjects.

MATERIAL AND METHODS

Two groups of subjects were studied. The normal individuals were ten subjects without blood or circulatory disorders. The second group consisted of ten patients with Starr Edwards ball valve prostheses, of whom seven had aortic and three mitral ball valves.

Blood sampling was done as non-traumatically as possible. 15–20 ml of blood were drawn into heparinized plastic syringes. The hematocrit was determined. Two plastic tubes were filled with 5 ml of blood each, centrifuged at $300 \times g$ for 10 min, the plasma was carefully collected and recentrifuged at $2\,000 \times g$ for 30 min. The plasma was analysed for total heme pigments by the benzidine method of Crosby and Furth (2), and for LDH activity according to the method of Wroblewski and laDue (13) Duplicate determinations were made.

Exactly 10 μl of blood were added to 2.0 ml of plasma from the same subject (dilution 1 200). Complete hemolysis was obtained by freezing at −20 C and thawing at room temperature three times. The heme content and the LDH activity were estimated in the hemolysate. By subtracting the values found in plasma and knowing the hematocrit, the content of LDH and pigments per ml of packed red cells could be calculated.

Ten ml of heparinized blood were transferred

Table I *Main hematological findings in 10 normals and 10 patients with prosthetic heart valves*

	Normals		Patients	
	Mean	S.D.	Mean	S.D.
Hb conc. (g/100 ml)	14.0	1.8	14.5	2.7
RBC (10^6/µl)	5.0	0.3	5.1	0.5
Hct ( )	42.0	3.3	42.0	2.7
Plasma LDH (U/l)	121	20	378	126
Plasma heme conc. (mg/100 ml)	5.4	1.6	8.5	5.0

to the cylindrical container of the hemoresistometer. The motor was started and turned the rotor at 3 000 rpm for 15 min. The rotor designated Hemolyse env. 35 mg% was used (3, 4). Blood samples were transferred from the container to plastic tubes, centrifuged and recentrifuged as described, and the plasma thus obtained was analysed for LDH and heme pigments. The differences between these values and the initial plasma values represented the loss of enzyme and pigment due to the mechanical trauma.

RESULTS

The main hematological findings are listed in Table I. The Hb concentration, RBC and Hct values were quite similar in the two groups of subjects studied. The degree of hemolysis was not particularly high in any of the patients as predicted from the plasma LDH activity. The highest LDH value recorded was 602 U/l (µmol/min/l) indicating an erythrocyte destruction rate of about twice the normal (10). None of the patients had developed hemolytic anemia.

The plasma Hb concentrations were moderately but significantly higher in the patient group ($p < 0.01$) exceeding the upper normal value in six patients.

It appears from Table II and Fig. 1 *a* that the LDH content of red blood cells, as calculated from the activity in the hemolysates, was considerably lower in patients with intravascular hemolysis than in normals. The difference is statistically highly significant ($p < 0.01$).

The total erythrocyte content, calculated in the same manner as the LDH activity, did not differ significantly between the two groups.

The loss of LDH and heme from erythrocytes during rotation in the hemoresistometer was calculated from the elevation of the plasma levels (Table III). In normals as well as in patients with ball valves the red cells were deprived of about twice as much LDH as heme pigments when subjected to the mechanical trauma, the differences being highly significant (Fig. 1 *b*).

The loss of heme pigments, reflecting the mechanical fragility of the red cells (3, 4), was quite similar in both groups of subjects. The degree of hemolysis produced by the hemoresistometer was low enough to allow comparison with the hemolysis occurring in patients with implanted ball valves.

DISCUSSION

The erythrocyte LDH activity in patients with prosthetic heart valves was markedly lower than that found in normal subjects, in spite of a relatively moderate degree of hemolysis. The erythrocyte content of heme pigments was normal in the patients with hemolysis. Further, in the patient group the plasma LDH activity was proportionally much more elevated than the plasma

Table II *The LDH activity and heme pigment content per ml packed red blood cells in normals and patients with heart valve prostheses*

	Normals		Patients	
	Mean	S.D.	Mean	S.D.
LDH activity (U/ml cells)	45.9	11.9	29.9	6.4
Heme pigments (mg/ml cells)	335	43	351	66

Table III *Loss of LDH activity and heme pigments from red blood cells to plasma during rotation in the hemoresistometer in normals and patients with prosthetic heart valves*

	Normals		Patients	
Loss from erythrocytes	Mean	S.D.	Mean	S.D.
LDH activity (10^{-4} U/ml cells)	75	36	72	48
Percent of total content	0.17	0.08	0.24	0.16
Heme pigments (10^{-4} mg/ml cells)	319	176	356	199
Percent of total content	0.09	0.05	0.10	0.06

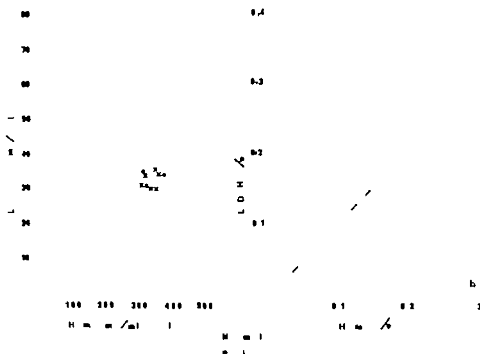


Fig. 1 (*a*) The erythrocyte content of LDH and heme pigments in normals and patients with intravascular hemolysis due to prosthetic ball valves.

(*b*) Loss of enzyme and pigment from blood cells during rotation of heparinized blood in the hemoresistometer pressed in % of the erythrocyte content.

heme concentration. These findings strongly suggest that erythrocytes subjected to mechanical trauma by the ball valve remain in the circulation in spite of a lowered content of LDH, that is, LDH is lost from morphologically undisrupted red blood cells. Our in vitro experiments support this hypothesis. When exposed to mechanical trauma in the hemoresistometer the erythrocytes lost much more of their LDH than of their heme pigments. The loss of pigments must be due to cell lysis, since the circulating erythrocytes of the patients had a normal heme content. Thus the proportionally much larger amount of enzyme given off must chiefly be derived from erythrocytes remaining in the circulation.

Green et al. (5) has found the activity of LDH and other glycolytic enzymes to be concentrated in the membrane fraction, which may in part explain the LDH loss from non-lysed erythrocytes during a mechanical trauma. Whether the five isoenzymes beha e differently in this respect has not been studied. The LDH values of normal erythrocytes correspond well with the findings of Wroblewski and laDue (13) who r the LDH activity of hemolysed whole blood to be approximately 100 times the serum value.

There was no definite correlation between erythrocyte content and plasma activity of the enzyme among our patients, but the group is too small and the degree of hemolysis too similar to allow definite conclusions on this point.

The present study clearly demonstrated that red blood cells lose LDH when subjected to mechanical trauma, and that the loss results in lowered LDH activity in the erythrocytes of patients with intravascular hemolysis due to prosthetic ball valves. These observations call for further investigation on the use of serum LDH determinations for predicting the degree of hemolysis in patients with heart valve prostheses.

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TECHNIQUE FOR SIMULTANEOUS CATHETERIZATION OF DIFFERENT PARTS OF THE PORTAL VEIN

PRELIMINARY REPORT

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The possibility to enter the portal system by reopening the umbilical vein in adult man was first reported by Gonzalez Carbalhaes in 1959 (1). After few attempts in earlier years we have used this way regularly since 1968. W soon found that with the aid of flexible steel guide wire it was possible to manipulate the catheter to all the various veins of the portal system (3). Although this considerably improved the means for study of the splanchnic organs, the single portal catheter did not allow detailed analysis of the splanchnic blood flow

In many patients with cirrhosis, long-standing biliary stasis, operative injuries to the hepatic vessels, splenomegaly etc there is evidence of altered flow distribution between the hepatic artery and portal vein or between the different parts of the venous supply to the main portal trunk. If these blood flows are to be measured separately simultaneous blood samples from different parts of the portal system are necessary W therefore developed method for placing three catheters in the portal system. One catheter was placed in the splenic vein for constant infusion of indicator or for blood sampling when the infusion was made in the splenic artery. The two other catheters were placed in the left and right intrahepatic branches of the portal vein. By simultaneous blood sampling from these two branches the complete mixing of indicator with blood from all parts of the venous supply to the portal vein could be checked, which is important for the flow determinations.

MATERIAL AND METHOD

Eight patients with overt or suspect abdominal disease were studied in connection to diagnostic portography. The umbilical cord as exposed extraperitoneally under local anaesthesia in the midline above the umbilicus. The cord was partly transected and probed into the distal end of the left intrahepatic portal branch.

In the first stage two catheters ere introduced into the portal vein and the wound as closed. A 40 cm long polythene catheter *PE 330* (2.92 3.73) mm as used as outer catheter and 75 cm long Ödman–Ledin polythene catheter (1.20/2.20 mm) as inner catheter The outer catheter was attached by an adapter to coax stopcock for coaxial catheters (Kifa, Stockholm, no 16 964) with d increased to 2.8 mm (A in Fig 1). The Ödman–Ledin catheter was introduced into the outer catheter through this coax stopcock, with its slightly bent tip straightened over the stiff part f 100 cm long stainless steel safety guide ire (d. 0 95 mm). A compressible O-ring at the entrance prevented leakage, but allowed movements between the catheters. Their dimensions were chosen not to *interfere with the free passage to the stopcock of the* outer catheter.

The double catheter system was introduced through the umbilical vein just to the distal end of the left intrahepatic portal branch. During TV-fluoroscopy the Ödman–Ledin catheter was successively manipulated to all the various veins of the portal system with the end of the flexible guide wire. This allowed injection of contrast medium for radiological serial examinations of the various parts of the portal system. After the X-ray investigation the Ödman–Ledin catheter was pulled out over the guide wire and replaced by 75 cm long polythene catheter *PE 205* (1.57/2.08 mm), placed in the splenic vein. T prevent portal thrombosis heparin in saline solution (20 000 IU in 1 000 ml) was used for slow infusion through both catheters. Prior to the flow study with additional catheters, 500 ml of 6% dextran solution (Macrodex®) was given.

In the second stage, few days later the PE 205 catheter in the splenic vein was replaced by 75 cm long teflon catheter (1.00 1.30 mm) over guide wire. By simultaneous attachment of the Y-piece (Kifa, Stockholm, no. 25 971 B in Fig 1), with adapters and compressible O-rings at both ends, to the coax stopcock of the outer catheter an additional 75 cm long teflon catheter (1.00/1.30 mm) could be introduced and placed in the right intrahepatic portal branch (Fig. 1).

The catheterization of the portal vein thus included

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Fig. 1. Catheter positions in the portal vein. *RPV* = right intrahepatic portal vein branch, *LPV* = left intrahepatic portal vein branch, *CV* = coronary vein, *SV* = splenic vein, *UV* = umbilical vein, *SMV* = superior mesenteric vein, *IMV* = inferior mesenteric vein, *A* = coax stopcock, *B* = Y-piece.

one small catheter in the splenic vein, another small catheter in the right intrahepatic portal branch and the outer catheter placed in the left intrahepatic portal branch at the junction of the umbilical vein.

The two portal vein catheters were used for blood sampling; the catheter in the splenic vein was used for infusion of indicator but could also be used for blood sampling (2). In t patients, however, the catheter could not be introduced into the splenic vein and was instead placed in the superior mesenteric vein and used for infusion of indicator.

RESULTS

It was more difficult to enter the main portal trunk ith double catheter system than ith the previously used single catheter. Insertion of the inner catheter past the portal main trunk was successful in six out of eight patients with different abdominal diseases. The proper placing of the catheters could be greatly facilitated by preforming also the oute catheter according to the presumed topography.

Three portal vein catheters were used in the four patients studied. All catheters could be properly placed, but in two patients only a few blood samples could be obtained from the outer catheter placed in the left intrahepatic portal branch. T enhance blood sampling the tip of the oute catheter has later been more thoroughly preformed and furnished with side holes.

Complications such as *intravascular clotting or local* injury to the portal vein were not recorded. After removal of the catheter it was, however, in one patient necessary to ligate a small bleeding artery in the abdominal wall.

CONCLUSION

Insertion of multiple catheters into the portal vein is possible by the present technique. As described in the subsequent paper (2), the catheters can be used to study blood f om different parts of the portal system simultaneously. Splenic vein blood flow can be measured simultaneously with the portal and hepatic vein flow if the splenic artery and a hepatic vein are also catheterized. A complete description of the present method will be given later.

ACKNOWLEDGEMENTS

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SIMULTANEOUS DETERMINATION OF PORTAL VEIN AND HEPATIC ARTERY BLOOD FLOW BY INDICATOR DILUTION TECHNIQUE IN AWAKE MAN

PRELIMINARY REPORT

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During operation the two separate afferent hepatic blood flows can be measured by electromagnetic flow meters placed around the portal vein and the hepatic artery. In awake patients, however, the precise determination of these two flows has not yet been possible. Only the total hepatic blood flow can be estimated, for instance by the Bradley technique with constant infusion of bromsulphalein or indocyanine green in a peripheral vein and measuring the hepatic arterio-venous difference of the substance.

In many patients with cirrhosis, long-standing biliary stasis, operative injuries to the hepatic vessels, etc., there is evidence of altered flow distribution between hepatic artery and portal vein, sometimes without any significant deviation of total hepatic blood flow. Therefore we thought it important to introduce a method to measure the portal and total hepatic blood flow simultaneously in awake patients. With a constant infusion of ^{133}Xe in the splenic artery or vein blood flows were calculated from the resulting xenon activities in portal and hepatic vein blood. By simultaneous blood sampling from the right and left intrahepatic portal branch the complete mixing of indicator in the portal vein could be checked, which is important for the flow determinations.

MATERIAL AND METHODS

The material comprises five patients with overt or suspect hepatic, biliary or pancreatic disease. The patients, who are listed in Table I, had no signs of porto-caval shunting at direct portography. The catheter was introduced in the portal vein via the reopened umbilical vein under local anesthesia. This portal catheter (i.d. 9? mm) was placed peripherally in the left intrahepatic portal branch at the junction of the umbilical vein. Through this catheter two others (1.0/1.3 mm) were introduced, one to the right intrahepatic portal branch and one to the splenic vein or the superior mesenteric vein (1).

The ideal situation for complete flow studies by indicator dilution technique would include four more catheters, one in the splenic artery, one in a peripheral artery, one in a right hepatic vein and one in a peripheral vein. No attempt was, however, made to insert all catheters in all patients, nor were all attempts successful.

An indicator dilution technique was used with constant infusion, according to Stewart, of 0.5–1.0 mC ^{133}Xe in 100 ml physiological saline and 5 % dextran. The indicator was infused at a rate of 4.9, 8.6 or 15.4 ml/min in the splenic artery, the splenic vein or the superior mesenteric vein for 6 to 20 min. After an equilibration period of a few minutes, air-free blood samples were repeatedly drawn from the peripheral artery, the right and/or left intrahepatic portal branch and the hepatic vein in heparinized 2 ml glass syringes. The ^{133}Xe activity of the samples was measured in a specially designed holder in front of a 3 inch NaI (Tl) crystal coupled to a Picker Digital Rate Computer. Assuming no significant portocaval shunting of blood the flows were calculated as

$$F_{portal\ vein} = \frac{F_{infusion}(C_{infusion} - \bar{C}_{portal\ vein})}{\bar{C}_{portal\ vein} - \bar{C}_{arterial}}$$

where F = flow in ml/min and C = activity of ^{133}Xe in counts/min/ml blood. The hepatic vein flow (e.g. portal vein flow + hepatic artery flow) was similarly calculated.

In four patients the total hepatic blood flow was simultaneously estimated by constant infusion of indocyanine green in a peripheral vein and measuring the plasma concentration in the artery and the hepatic vein after equilibration.

RESULTS

During constant infusion of ^{133}Xe the arterial background was low and constant due to exhalation of most of the gas during the first passage through the lung capillaries. With infusion in the splenic vein constant values for xenon activity in the portal vein were obtained already 1–2 min after the start of infusion. The time for equilibration of xenon activity between the perfusing blood and the liver, and thus for obtaining constant values in the hepatic vein, was about 3 min with infusion in the

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Table I *Main data of the patients*

Pat. no.	Age (y)	Sex	History	PBI (.../l/24 h)	CR (/24 h)	PBI (µg/100 ml)	BEI (µg/100 ml)	^{131}I uptake (.../24 h)	Remarks
1	49		Subtotal thyroidectomy for non-toxic goitre 30 y ago		69	5.8	3.9	14	Plum-sized thyroid remnant
2	36		*Infectious thyroiditis 1 y ago*	*0.47*		6.3	4.7	33	Thyroid not palpable
3	60		Radioiodine treatment 15 mo ago (8 mCi)	1.19		4.6		20	Thyroid not palpable
4	36		Subtotal thyroidectomy for thyrotoxicosis 12 y ago	0.37		6.1		65	Hard nodule about 50 g, other thyroid tissue not palpable
5	22		Infectious thyroiditis 7 mo ago	0.22		4.9	1.8	50	Thyroid tissue about 80 g, no nodules
6	5.		Subtotal thyroidectomy for thyrotoxicosis 10 y ago			3.8		52	Thyroid not palpable
7	64		Subtotal thyroidectomy for thyrotoxicosis 18 y ago			6.0		29	Left lobe firm, about 2 cm in diameter
8	44		Subtotal thyroidectomy for *thyrotoxicosis 16 y ago and for relapse 8 y ago*			5.5			Remaining tissue hard, about *1.5 cm* in diameter

thyroid's functional capacity is used, and only small amounts of hormones are secreted, i.e. both the fractional and mg turnover are small.

In some instances the fractional and mg turnover rates do not run parallel.

MATERIAL AND METHODS

...ght patients are presented, all of whom showed symptoms and signs ...hich are usually attributed to hyperthyroidism (7). These ...ere perspiration, palpitation, ...eight loss, nervousness, fatigue, intolerance to heat, dyspnoea, insomnia, tachycardia and moist and warm skin. The main features of the patients are compiled in Table 1. Patients 1–5 all show the same pattern; the functional thyroid mass has been diminished by operation, radioiodine or thyroiditis, and the PBI and/or butanol-extractable iodine (BEI) in serum is normal or low. In contrast to this, the PBI and/or CR are high. Patients 6, 7 and 8 resemble the former but PBI and CR ...alues are not available.

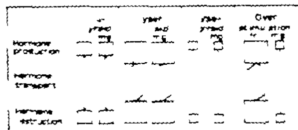


Fig. 1 Schematic representation ...f thyroid hormone pathways in different conditions. *fr* Fractional values, *mg* mg values, (see text).

The 24-hour radioiodine uptakes by the thyroid vary and are low in patients 1, 3 and 7, normal in patient ..., elevated in patients 4, 5 and 6, and not available in patient 8.

When the patients were treated with thyroxin, the symptoms and signs subsided. The thyroxin doses varied from 100 to .50 µg/day with ...starting dosage of 100 or sometimes 50 µg/day.

DISCUSSION

If the thyroid's hormone manufacture is diminished by any means, then pituitary TSH secretion increases. This is true both in euthyroidism and in hyperthyroidism (4). If almost, but not all, thyroid function capacity is eliminated by surgery or irradiation, or as a consequence of thyroiditis, the thyroid hormone pool of course is diminished. When pituitary TSH is secreted, the functioning thyroid remnant uses its utmost capacity to secrete hormone, i.e. the fractional turnover increases and the PBI and CR rise. This was reported as early as 1951 (1) to occur after subtotal thyroidectomy and also after thyroiditis (2) and after radioiodine treatment (3). In contrast to this high activity the resulting net hormone production is small because the thyroid remnant is capable of secreting only minute amounts of hormone. Thus the mg rate (and hormone values in blood) remains low in spite of the high fractional rate (and high PBI and CR).

The whole functioning thyroid tissue is working for hormone synthesis and secretion. To achieve this, the peripheral destruction of hormones also increases. Hormones are metabolized and iodine recirculated at a rapid rate, although the amount of hormones present per unit of time in any location or compartment is small. This situation may be called "the overstimulated thyroid remnant" or "the overstimulated thyroid". These concepts are illustrated in Fig. 1.

A similar situation may be seen in subjects incorrectly treated with antithyroid drugs (11). In such patients the radioiodine uptake by the thyroid is elevated. An elevated uptake is seen in patients 4, 5 and 6 in the present report. The *variability of the radioiodine uptake values may* be explained by the rapid iodine turnover in the thyroid. Thus, in some patients, the uptake maximum is without doubt reached at a considerable time before the 4-hour measurement is done. Incidentally the patients with especially high PBI or CR values are the ones with low uptake values, which may speak in favour of this explanation.

It is, of course, a matter of opinion whether patients 6, 7 and 8 in whom PBI or CR measurements are not available, belong to the same category as the other five patients. The hyperthyroid symptoms and signs, normal PBI and favourable response to thyroxin medication seem to justify their place in this group.

It is interesting that patients with an overstimulated thyroid show symptoms and signs of hyperthyroidism although the condition must in fact be interpreted as a form of hypothyroidism. This has been reported previously (5, 6, 9). These symptoms and signs subside when thyroxin medication is started. It has been suggested (10) that the clinical picture of hyperthyroidism could be the result of an increase in destruction rate of thyroid hormones in body cells, and not necessarily of increased amounts of hormones secreted and circulated. Observations on overstimulated thyroid patients may corroborate this view. Hormone degradation studies, as well as short-time turnover studies, may be helpful in shedding light on the situation.

The clinical consequence of these findings is that hyperthyroid symptoms and signs may actually mean that hypothyroidism is present, and should be treated by thyroid replacement. It is especially important to recognize these patients as not having a relapse of hyperthyroidism. These observations also emphasize the importance of a thorough follow-up of patients treated by subtotal thyroidectomy or with radioiodine, and of patients who have had thyroiditis. Early replacement with thyroxin during follow-up does, of course, eliminate the risk of an overstimulated thyroid in these patients.

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Table I *Selected clinical data*

SR = sinus rhythm, NR = nodal rhythm, CSR = coronary sinus rhythm, AF = atrial fibrillation, AFl = atrial flutter VEB ventricular ectopic beat, *SVEB* = supraventricular ectopic beat, *RBBB* = right bundle branch block, LAHB left anterior hemiblock, A-arrest = atrial arrest, MI = mitral insufficiency MS = mitral stenosis, AI = aortic insufficiency AS aortic stenosis, CMP = cardiomyopathy LAE = left atrial enlargement, LVH = left ventricular hypertrophy NM = not measured

Case no.	Age (y)	Sex	Diagnosis	Heart vol.	P_{PA} (mmHg) syst./diast. (mean)	P_{RA} (mmHg) mean (*a* wave)	ECG	Earlier arrhythmias	Electrolyte abnormalities
1	36	♂	MI	920/560	17/4 (9)	0 (3)	SR LAE LVH	0	0
5	29	♂	AS	680/330	24/4 (11)	2 (3)	SR	Rare SVEB	0
7	44	♂	MS AI	1 000/490	31/8 (17)	0 (3)	SR	0	0
8	61	♂	MS	650/400	RV 38/4	3 (9)	SR LAE	AF 3	0
9	48	♀	MI	770/420	24/9 (14)	2 (5)	SR	(Arrhythmia feeling)	0
11	33	♂	Long AF + dig. intox.	1 020/550	15/5 (9)	2	NR RBBB LAHB A-arrest	AF A-arrest	0
14	56	♂	MI?	1 080/570	27/12 (18)	6 (9)	SR LVH	0	0
16	54		CMP	1 050/610	32/15 (23)	2 (3)	SR	Sinus tachyc.	0
18	64	♂	AS	1 160/580	24/10 (17)	2 (5)	SR SVEB VEB LVH	SVEB VEB	0
19	27	♂	AS	730/350	21/8 (15)	3 (4)	SR	0	0
20	50	♂	AS	750/400	22/9 (13)	1 (5)	SR	0	0
21	28	♂	Innoc. murm.	1 090/420	18/5 (12)	1 (2)	SR	0	0
22	44	♂	Chron. VEB	620/330	17/8 (11)	2 (3)	SR VEA	VEB (tachyc. feeling)	0
23	35	♀	AS	1 000/600	29/12 (18)	4 (9)	AF→SR LVH	Sinus tachyc.	0
24	41	♂	Constr peric.	Not measurable	38/13 (21)	10 (13)	CSR	VEB	0
25	41	♂	Constr peric.	1 100/500	19/12 (15)	11 (14)	SR nonspec. ST T changes	0	Ca, P NM
28	50	♂	AI MI	1 630/880	32/18 (24)	8 (15)	SR A-V block I LVH	VEB A-V block I	0
29	47	♂	CMP	1 240/670	RV 78/13	13	NR A-arrest	AF NR VEB A-arrest	0
31	59	♂	AI AS	850/490	15/5 (9)	1 (2)	SR LVH	0	Ca, P NM
32	39	♀	Heart neurosis	580/310	18/9 (13)	4 (6)	SR	0	0
33	24		Peric. calc.	560/340	23/14 (19)	6 (9)	SR VEB	VEB	0
34	39	♂	AS AI	720/400	26/11 (17)	3 (6)	SR	0	0
35	45	♂	AI MI	1 140/540	22/8 (12)	1 (3)	SR LVH	(Arrhythmia feeling)	0
38	54	♂	AI	1 250/630	49/20 (30)	4 (7)	SR LVH	0	Ca, P NM
39	44	♂	AI	1 450/780	32/12 (22)	1 (3)	SR LVH	VEB SVEB	0
40	55	♂	AS AI MS?	1 280/670	43/17 (67)	3 (5)	SR LAE LVH	VEB	0
42	58		MS	800/470	39/17 (26)	4 (7)	SR LAE LVH	AF	0
43	23	♂	AS	450/260	RV 20/3	4	AFl	AS	0

Selected clinical data from all patients are given in Table I. The patients are numbered according to their consecutive order in the series of MAP recordings in the laboratory. The investigation and MAP recording were performed in the same way as described elsewhere (25). Seventy-three right ventricular MAP recordings meeting the requirements for an acceptable recording (26) were obtained during regular spontaneous heart rhythm in supine position from 27 of the 28 patients. Sixteen patients were free from digitalis treatment, 15 of them being in sinus rhythm and one in nodal rhythm. These patients will be referred to as the digitalis-free group. The acute effect of 1.2 mg Lanatoside C i.v. upon the right ventricular MAP was studied in four of these patients. In two other patients in sinus rhythm and in the patient in nodal rhythm the acute effect of 0.5 mg atropine i.v. was studied. Right ventricular MAP was studied during regular rhythm in ten patients with chronic digitalis and no quinidine treatment, seven of them being in sinus rhythm, one nodal rhythm, one coronary sinus rhythm, and one in regularly blocked atrial flutter. These patients will be referred to as the digitalis-treated group. In addition right ventricular MAP was studied in one patient with sinus rhythm and on chronic quinidine as well as digitalis treatment.

The analysis of single MAP was carried out in the same way as earlier described for an atrial MAP (25). In addition the QT time and QRS duration were measured from the simultaneously recorded ECG. All results are the means values of calculations performed from five

heart cycles during which the cycle length has not been allowed to deviate more than ±10% from the mean value. This limitation has been used as the criterion for "regular rhythm". The analysis of a single MAP is schematically depicted in Fig. 1.

Fourteen right ventricular MAP recordings acceptable for analysis, at different regular rates, were obtained during electrical pacing of the right atrium at the entrance of the superior vena cava with stepwise increasing pacing frequency up to 150/min. The pacing was performed with an external pacemaker (Elema 138 or American Optical 10970 R) using a bipolar pacemaker catheter (USCI 5652) introduced from a brachial vein. The pacing was maintained during at least ten heart beats at the different regular rates. The MAP analyses were performed from the last five beats. In two patients the right ventricular MAP was recorded during right atrial pacing before and after digitalis infusion. The right ventricular MAP could not be analysed during spontaneous regular rhythm immediately prior to the pacing-induced heart rate increase in one of the 14 recordings (case 42).

RESULTS

Spontaneous regular heart rhythm

The results from the analyses of all the recordings during spontaneous regular heart rhythm are presented in Table II. Due to the completely rounded contour of phase 2 in some recordings no distinction could be made between phases 1–2 and 3 of the MAP. In these recordings, therefore, no estimation of the duration of phases 1–2 and 3 has been possible. Examples of right ventricular MAPs of the different types are depicted in Fig. 2.

The amplitudes of all recordings from the right ventricle during spontaneous heart rate range between 10.6 and 51.6 mV (mean value 25.7 mV, S.D. 8.0 mV). The amplitude difference between repeated recordings in an individual patient ranges between 0 and 29.9 mV.

The time from the onset of the QRS complex of the unipolar right ventricular electrogram to phase 0 of the MAP (Q-phase-0 interval) ranges between 0 and 67 msec in all recordings during regular rhythm and in the absence of right bundle conduction defects. In two patients right ventricular MAP recordings were obtained in the presence of right bundle branch block. The Q-phase-0 interval ranges between 45 and 56 msec in these recordings.

The duration of phase 0 of the right ventricular MAP ranges between 2 and 20 msec in all recordings, and the total duration of the MAP

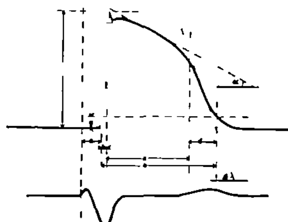


Fig. 1. Analyses of right ventricular MAP. Top tracing = right ventricular MAP. Lower tracing = right ventricular electrogram. – Q-phase-0 interval, *b* = duration of phase 0, – duration of phases 1–2, *d* = duration of phase 3, – total duration of MAP, *f* = amplitude of MAP, *g* = *h* = *f*/10, [tg α] *i* = relative repolarization rate (RRR) of phase 2, [tg β] *j* = relative repolarization rate (RRR) of phase 3. Time intervals given in msec, amplitudes in mV and RRRs in %/sec.

between 203 and 364 msec. The difference in total MAP duration in repeated recordings in an individual patient varies between 0 and 38 msec under unchanged pharmacological conditions and at heart rates within ±10% of their mean value. The variation coefficient for the duration of all MAP recordings under these circumstances in the individual patient ranges between 0.6 and 9.8% (median value 3.4%).

The duration of the right ventricular MAP increases with increasing cycle length in the digitalis-free group. The relationship is less evident in the digitalis-treated group (Fig. 3). The presented values are means of different recordings if more than one recording has been made in an individual patient and the criterion for regular rhythm has been fulfilled. The values from the analyses of the MAP after acute administration of digitalis or atropine are not included in the figure. The equation for a mean square fitted regression line in the digitalis-free group is $Y = 0.13\,X + 166.5$ (= 0.76, S_{yx} = 21.3, $\bar{y}$ = 277.8) and in the digitalis-treated group $Y = 0.01\,X + 263.3$ (= 0.02, S_{yx} = 42.0, $\bar{y}$ = 268.0).

The relative repolarization rate (RRR) of phase 3 increases with decreasing duration of the right ventricular MAP (Fig. 4). The regression

Table II *Results from analyses of right ventricular MAP during regular spontaneous rhythm*
Abbreviations, see Table I

Rec. No.	Rhythm	Drug	Q-ph0 (msec)	Cycle length (msec)	Ampl. MAP (mV)	MAP duration (msec) Total (ph0+ph1 2+ph3)	RRR ph2 (%/sec)	RRR ph3 (%/sec)	QT (QRS) (msec)
1A	SR		0	703	33 7	239 (7+160+72)	271	779	340 (90)
1C	SR		10	725	28.4	243 (5+167+71)	228	792	340 (90)
5C	SR		27	638	21.6	234 (5+156+73)	207	932	320 (100)
5D	SR		12	618	28.3	249 (8+168+73)	239	857	320 (100)
7A	SR		36	917	30.5	269 (7+182+80)	191	746	345 (90)
8C	SR	Dig.	13	880	31 1	264 (7+177+80)	287	787	340 (80)
9B	SR	Dig.	22	658	23 7	227 (10)		659	315 (80)
9C	SR	Dig.	50	693	25.4	236 (10)		652	320 (80)
11A	NR+RBBB +LAHB	Dig.	45	1 057	23.0	220 (10)		744	380 (160)
11B	NR+RBBB +LAHB	Dig.	50	1 064	24.8	240 (6)		665	380 (160)
11C	NR+RBBB +LAHB	Dig.	53	1 069	26.6	240 (10)		691	400 (160)
11D	NR+RBBB +LAHB	Dig.	56	1 048	21.9	233 (5)		672	395 (160)
14A	SR		55	1 122	22.2	305 (10)		683	490 (90)
14B	SR		47	1 151	23.4	305 (11+204+90)	206	650	440 (90)
14C	SR		63	1 148	24.6	308 (8)		660	450 (90)
14D	SR	Dig.	53	1 190	19.0	294 (7)		600	425 (90)
16B	SR	Dig.	5	756	17.2	364 (5+254+105)	213	366	450 (80)
16D	SR	Dig.	12	724	20.3	327 (8+218+101)	238	511	450 (80)
16E	SR	Dig.	9	706	10.6	326 (9+212+105)	216	540	450 (80)
18C	SR	Dig.	29	886	29.5	299 (6+198+ 95)	232	614	390 (110)
19A	SR		15	793	20.7	262 (20+162+ 80)	220	726	345 (100)
19D	SR		29	821	25.6	247 (18)		726	355 (100)
19E	SR		18	818	33 1	268 (14+163+ 91)	232	701	355 (100)
20B	SR		48	761	23.5	250 (7)		932	370 (80)
20C	SR		50	859	15.6	253 (5)		939	340 (80)
20D	SR		53	720	23.3	238 (16)		863	350 (80)
20E	SR		53	774	32.5	254 (6+162+ 86)	205	790	340 (80)
20G	SR	Dig.	37	753	32.4	235 (12+141+ 82)	247	801	360 (80)
21B	SR		25	960	35 7	307 (9+210+ 88)	205	635	400 (80)
21E	SR	Atr	34	702	22.9	249 (9+170+ 70)	271	761	340 (80)
21G	SR	Atr.	30	665	21.5	253 (4)		988	340 (80)
22C	SR		40	726	22.4	269 (10)		897	370 (80)
23B	SR+RBBB[a]	Dig.	45	654	16.6	244 (14)		876	400 (145)
24B	CSR	Dig.	12	873	20.0	310 (13+181+116)	234	516	380 (90)
24C	CSR	Dig.	21	872	18.4	291 (5+171+115)	220	578	370 (90)
25B	SR		44	861	22.6	257 (8+163+ 86)	207	734	
25C	SR		67	881	13 1	266 (4+181+ 81)	123	855	
25E	SR		22	871	32.2	285 (14+190+ 81)	236	660	
25F	SR	Atr	32	690	32.4	249 (12 152+ 85)	302	636	
25G	SR	Atr	35	606	12.9	241 (17+139+ 85)	301	609	
25H	SR	Atr	67	627	22.2	237 (18)		671	
28B	SR+A V bl. I		13	700	21 7	300 (6)		602	
28D	SR+A-V bl. I		14	709	51.6	309 (16+174+119)	205	484	
28E	SR+A-V bl. I		27	734	47.8	297 (5+181+111)	190	524	
28F	SR+A V bl. I		12	679	30.3	286 (5+168+113)	211	534	
29A	NR		22	1 332	30.1	364 (11+257+ 96)	128	535	510 (90)
29B	NR	Atr	20	1 165	20.2	335 (8+259+ 68)	125	434	485 (90)

Table II (*continued*)

Rec. No.	Rhythm	Drug	Q-ph0 (msec)	Cycle length (msec)	Ampl. MAP (mV)	MAP duration (msec) Total (ph0+ph1 2+ph3)	RRR ph2 (%/sec)	RRR ph3 (%/sec)	QT (QRS) (msec)
31A	SR	Dig.	17	1 081	27.6	277 (5+175+ 97)	197	597	360 (90)
31C	SR	Dig.	38	1 003	14.5	241 (3)		673	380 (90)
32B	SR		10	714	21.6	298 (18+183+ 97)	199	512	405 (80)
32C	SR		35	760	14 9	302 (12)		575	430 (80)
32D	SR	Dig.	30	765	16 5	270 (12+153+105)	157	533	395 (80)
32E	SR	Dig.	23	734	15.2	278 (13+173+ 92)	178	545	395 (80)
32F	SR	Dig.	7	767	16.7	285 (6+189+ 90)	223	580	380 (80)
32G	SR	Dig.	18	751	19 1	273 (13+162+ 98)	191	522	385 (80)
32H	SR	Dig.	18	763	14.6	279 (18+158+103)	219	552	375 (80)
33A	SR		23	742	28 8	258 (3+178+ 77)	247	652	360 (90)
33C	SR	Dig.	27	809	25.1	247 (7+146+ 94)	135	736	370 (90
34B	SR		51	921	24 6	284 (8)		766	390 (100)
35D	SR		20	898	34.2	255 (12)		635	350 (100)
38B	SR		18	790	32.4	302 (7+222+ 73)	219	705	365 (100)
38C	SR		43	690	27.8	274 (2+194+ 78)	134	815	370 (100)
38D	SR		40	672	29 6	275 (9+197+ 69)	194	795	370 (100)
38E	SR		53	732	36.0	269 (10+178+ 81)	108	809	370 (100)
38G	SR		7	655	36 4	281 (9+194+ 78)	201	727	370 (100)
39B	SR	Dig	23	765	44.2	299 (8+187+104)	166	584	420 (130)
39C	SR	Dig.	9	769	36.5	295 (7)		657	420 (130)
39D	SR	Dig	8	799	37 4	295 (11+190+ 94)	143	668	420 (130)
40C	SR	Dig. + quin.	12	870	27 1	284 (8+155+121)	213	517	
43A	AFl	Dig.	47	702	31.5	203 (10)		778	295 (80)
43B	AFl	Dig.	41	699	24.9	222 (12)		824	295 (80)
43C	AFl	Dig	50	699	25.7	216 (8)		815	295 (80)
43D	AFl	Dig.	49	700	24 8	211 (10)		788	290 (80)

During the investigation.

has been calculated on the same principles as in Fig. 3. The equation for the regression line in the digitalis-free group is $Y = -2.59\ X + 1433.1$ ($r = 0.69$ $S_{yx} = 89.9$ $\bar{y} = 713.0$), and for the digitalis-treated group $Y = -2.37\ X + 1306.9$ ($r = 0.77$ $S_{yx} = 82.6$, $\bar{y} = 671\ 7$).

The RRR of phases 1–2 could be calculated from at least one recording in 13 of the 16 patients in the digitalis-free group. In the digitalis-treated group, analyses of RRR of phases 1–2 could be performed in six patients. A decreasing MAP duration is accompanied by an increased RRR of phases 1–2 (Fig. 5). The equation for the digitalis-free group is $Y = -0.71\ X + 400.8$ ($r = 0.73$, $S_{yx} = 23\ 4$ $\bar{y} = 202.3$) and for the digitalis-treated group $Y = -0.47\ X + 360.4$ ($r = 0.28$ $S_{yx} = 46.6$, $\bar{y} = 220.0$)

The duration of phases 1–2 and 3 both vary with the duration of the individual MAP (Fig. 6). The equation for the linear relationship between the duration phases 1–2 and the total duration of the MAP is $Y = 0.72\ X - 19\ 1$ ($r = 0.91$ $S_{yx} = 10.7$). The duration of phase 3 is related to the total duration of the MAP according to the equation. $Y = 0.27\ X + 13.5$ ($r = 0.66$, $S_{yx} = 9\ 8$) The recordings from the digitalis-free and digitalis-treated groups have not been separated in these calculations, as the relationships in the two groups were very similar and no statistical evidence of differences between the groups existed.

When the mean value of the duration of the right ventricular MAP during regular rhythm is related to the mean value of the QT time from the simultaneously recorded ECG the relationship depicted in Fig. 7 is found. The values from patients 25 28 and 40 could not be included in this presentation due to inability to define the QT interval. The figure also presents the cor

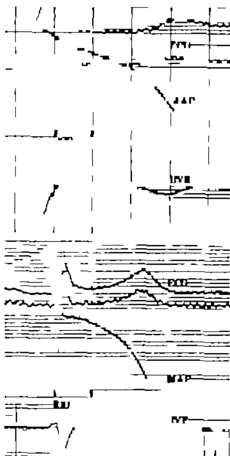


Fig. 4. Different types of right ventricular MAPs. Recording *a* exhibits distinct level corresponding to phase 2, allowing calculation of RRR of this phase and subgrouping of the total duration of the MAP in duration of phases 0, 1 2 and 3 (case 29). Recording *b* has no distinct plateau and thus no distinct tangent of phases 1 2 can be constructed (case 21) This makes calculation of RRR of this phase as well as distinction between the durations of the different phases impossible.

responding relationship when the QRS time has been subtracted from the QT time. The equation for the relationship between the QT time and the duration of the MAP in all cases except those with RBBB is $Y = 1.33\ X + 9.2$ ($r = 0.95$). When correction for QRS time has been made, the equation for the regression line in all cases is $Y = 1.27\ X - 64.6$ ($r = 0.92$)

In the four patients who were studied before and after 1.2 mg Lanatosid C i.v. the duration of the right ventricular MAP decreases in every patient. The RRR of phase 3 shows no uniform tendency to change after acute digitalization. The effect of acute digitalization upon the RRR of phases 1–2 cannot be assessed, as phases 1 2 could only be defined in a few recordings. Thus it has not been possible to evaluate the effect of acute digitalization upon the duration of phases 1–2 or of phase 3 either

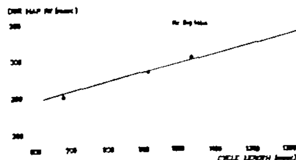
Acute atropine injection is followed by a decreased duration of cycle length and MAP duration in the three patients investigated before and after the administration of the drug.

No relationship has been found between ECG diagnosis, heart volume, pulmonary artery pressure, right atrial pressure and amplitude, duration, RRR of phases 1–2 or 3 of the right ventricular MAP

Pacing-induced regular heart rhythm

During atrial pacing with stepwise increasing frequency there is regularly a prolongation of the P-Q time. In five pacing experiments an A V block II appears at high pacing rate.

The correlation between the duration of the right ventricular MAP and the cycle length (RR) during stepwise increase of heart rate via right atrial pacing has been tested for fitness of



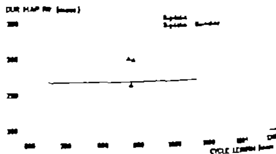
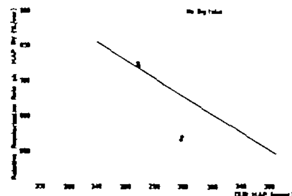


Fig. 5. Relationship between cycle length and duration of right ventricular MAP The result from the patient treated with both digitalis and quinidine is not included in the regression analyses. For further explanation see text.



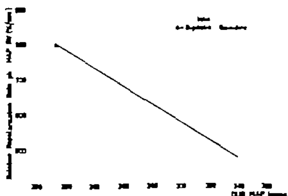
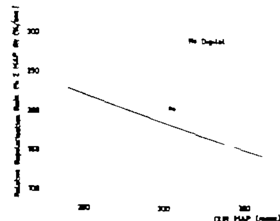


Fig. 4. Relationship between RRR of phase 3 and duration of right ventricular MAP during spontaneous regular rhythm. The result from the patient treated with both digitalis and quinidine is not included in the regression analysis. For further explanation see text.

linear logarithmic, square root and cube root equations. The correlation coefficient varied in the different functions between 0.951 and 0.999 (Table III). None of the functions had any obvious superiority over the others. The linear relationship between the duration of right ventricular MAP and the RR in all pacemaker experiments is depicted in Fig. 8. The effects of atrial pacing upon the right ventricular MAP in the patients treated with digitalis do not differ systematically from those of the digitalis-free patients.

The duration and RRR of phases 1–2 could be calculated in only 8 of the 14 successful recordings performed during variation of cycle length by atrial pacing. Furthermore, in some of these 8 recordings, it has only been possible to distinguish between phases 1–2 and 3 when the total duration of the MAP was relatively long, e.g. at long cycle lengths. In these recordings, however the shortening of the right entricular MAP is predominantly due to a shortening of phases 1–2 (Fig. 9) There is no obvious general shortening of phase 3 in the corresponding recordings. The variation in rate of repolarization of phases 1–2 xhibits no uniform trend. Nor does the RRR of phase 3 in the 14 recordings show any general tendency to increase concomitantly with shortening of the duration of the right ventricular MAP induced by atrial pacing (Fig. 10)



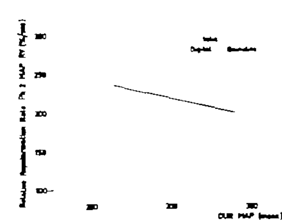


Fig. 5. Relationship between RRR of phase 2 and duration of right ventricular MAP during spontaneous regular rhythm. The result from the patient treated uth both digitalis and quinidine is not included in the regression analysis. For further explanation see text.

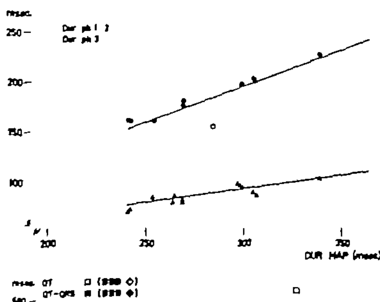


Fig. 6 Relationship between duration of different phases and total duration of right ventricular MAP during spontaneous regular rhythm. The regression line is calculated from both the digitalis-free and the digitalis-treated group but does not include the patient with both digitalis and quinidine. O Δ = the patient with both digitalis and quinidine.

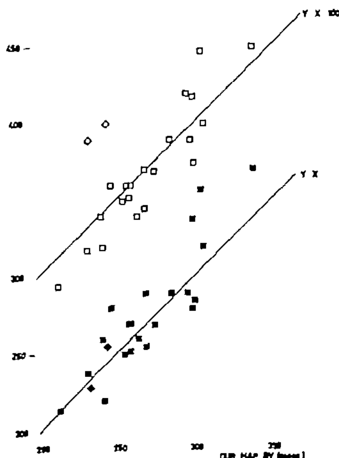


Fig. 7 Relationship between QT time, QT minus QRS time and duration of right ventricular MAP during spontaneous regular rhythm. In cases 11 and 23 there is an obvious prolongation of the QT time compared with the duration of the MAP. Both these patients had RBBB during the investigation and, when correction for the QRS duration has been made, the relationship fits with that found in the other patients. The line of identity and the line $Y = X + 100$ are included for comparison.

DISCUSSION

A MAP recording gives no information about the total amplitude of the electric phenomenon of a single cell, as does a microelectrode recording of the AP (11). Thus the overshoot and resting membrane potential of an AP from a single cell cannot be interpreted from a MAP recording (11). Neither can the MAP give a true reproduc

Table III *Regression coefficients between duration of right ventricular MAP and functions of RR*

Rec. no.	$X - RR$	$X - \log RR$	$X - RR^{1/2}$	$X - RR^{1/3}$	< RR <
Without digitalis					
14 B	0.946	0.984	0.969	0.975	428–1151
20 D	0.995	0.997	0.998	0.998	382– 720
21 G	0.985	0.977	0.986	0.987	405– 665
28 F	0.963	0.960	0.962	0.962	606– 814
31 A	0.982	0.968	0.986	0.987	602– 827
34 B	0.966	0.988	0.981	0.985	405– 921
35 D	0.973	0.987	0.982	0.984	407– 898
38 G	0.999	0.998	0.997	0.996	407– 655
With digitalis					
18 C	0.971	0.967	0.967	0.966	607– 886
20 G	0.990	0.997	0.995	0.996	380– 753
31 C	0.994	0.985	0.991	0.989	607–1003
32 E	2 heart rates				608– 734
33 C	0.977	0.951	0.967	0.962	423– 809
42 D	2 heart rates				404– 485

tion of the electrical events during phase 0 (11, 25). However, with proper recording technique, the repolarization course of the MAP is a very good representation of this event as recorded with the microelectrode technique in close vicinity to the MAP recording site (11). This permits comparisons between acceptable MAP recordings and APs with respect to duration and relative changes of the repolarization course. The criteria of an acceptable MAP recording applied in the

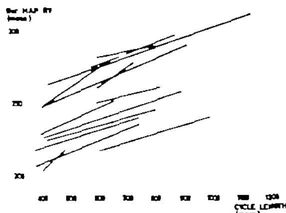


Fig. 8. Relationship between duration of right ventricular MAP and RR during different regular heart rates induced by atrial pacing. The equations for the lines are:

Rec. No	Eq.	Rec. No.	Eq.
14 B	$Y = 0.07\,X + 234.6$	32 E	$Y = 0.13\,X + 179.0$
18 C	$Y = 0.06\,X + 246.8$	33 A	$Y = 0.05\,X + 223.5$
20 D	$Y = 0.06\,X + 195.0$	33 C	$Y = 0.05\,X + 201.5$
20 G	$Y = 0.08\,X + 177.7$	34 B	$Y = 0.08\,X + 214.0$
21 G	$Y = 0.09\,X + 193.9$	35 D	$Y = 0.06\,X + 199.8$
28 F	$Y = 0.04\,X + 259.0$	38 G	$Y = 0.13\,X + 194.5$
31 C	$Y = 0.06\,X + 183.2$	42 D	$Y = 0.17\,X + 134.2$

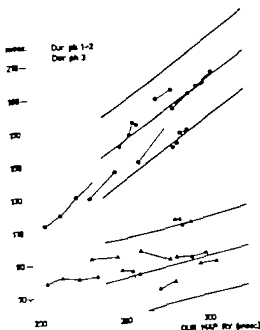


Fig. 9. Effect of pacing-induced variation of right ventricular MAP duration upon duration of the different phases. The figure also shows the regression lines and 95% confidence limits from results of similar analyses of MAP recordings during spontaneous regular rhythm from the remaining patients in the digitalis-free and digitalis-treated groups.

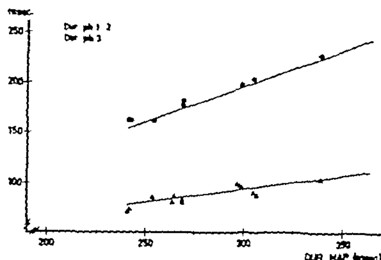


Fig 6. Relationship between duration of different phases and total duration of right ventricular MAP during spontaneous regular rhythm. The regression line is calculated from both the digitalis-free and the digitalis-treated group but does not include the patient with both digitalis and quinidine. ○ △ = the patient with both digitalis and quinidine.

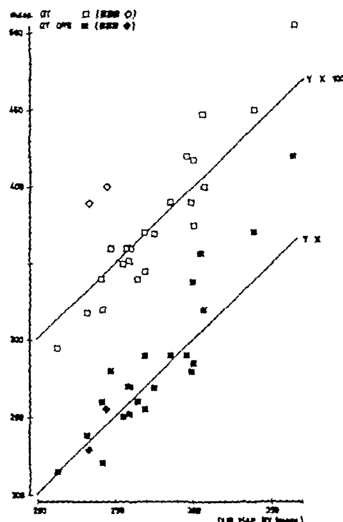


Fig 7. Relationship between *QT* time, QT minus QRS time and duration of right ventricular MAP during spontaneous regular rhythm. In cases 11 and 23 there is an obvious prolongation of the QT time compared with the duration of the MAP. Both these patients had RBBB during the investigation and, when correction for the QRS duration has been made, the relationship fits with that found in the other patients. *The line of identity* and the line $Y = X + 100$ are included for comparison.

DISCUSSION

A MAP recording gives no information about the total amplitude of the electric phenomenon of a single cell, as does a microelectrode recording of the AP (11). Thus the overshoot and resting membrane potential of an AP from a single cell cannot be interpreted from a MAP recording (11). Neither can the MAP give a true reproduc-

rate in this patient group in the same way as the rate dependence of the AP MAP and QT durations in the cited studies.

The duration of the QT time has, amongst other factors, been shown to exhibit variations with sex, ionic composition of the blood, digitalis and quinidine therapy (18) Of these factors only the effect of digitalis is of such magnitude that it can be interpreted with reasonable assurance having regard to the small number of patients.

Acute digitalization causes a heart rate decrease. This fall of heart rate will cause prolongation of the duration of the MAP However the over-all effect of digitalis in this study is a reduction of heart rate and a shortening of the right ventricular MAP duration. This means that the pure effect of digitalis upon the right ventricular MAP is a more pronounced shortening than that observed in connection with the concomitant negative chronotropic effect. Elsewhere the shortening of the ventricular MAP induced by digitalis was attributed to an increased repolarization rate during phase 2 (29) However the change of the ST segment of the ECG might have interfered with this phase (26) Ouabain in physiological doses causes a slight reduction of the duration of the AP recorded from single ventricular muscle cells in specimens of mammalian hearts (23) From the figures reported the shortening seems to be due to a shortened phase 2, which also exhibits a faster repolarization rate. After a long time of exposure the duration of the AP becomes markedly shortened in spite of a decreased repolarization rate, as regards phase 3 (23).

The configuration of the MAP recording from the patient on both digitalis and quinidine therapy is in agreement with the observation of the action of quinidine on the AP of isolated hearts (7). There is a slowing of the repolarization of phase 3 with a prolongation of this phase causing a prolongation of the total duration of the AP Further investigations need to be performed in order to see whether the observation in the present patient is of general validity

In the three patients who received atropine, the duration of the right ventricular MAP decreased concomitantly with the increasing heart rate. The pure effect of vagal stimulation upon the dog ventricular AP duration is negligible (27). During vagal discharge and a constant heart rate, atropine causes a minimal prolongation of the dog ventricular AP duration, not at all of the same magnitude as the variations caused by a change in heart rate (27) Thus the decrease of the duration of the right ventricular MAP in the three patients who received atropine is almost certainly due to the increased heart rate induced by the vagal inhibition.

The variability in the duration of ventricular APs within a particular species is pronounced (10) No reports are available on the influence of the repolarization rates and durations of the different phases on the total duration of the ventricular AP or MAP during spontaneous rhythm in animal groups of any mammalian species. In the present patient group the prolongation of the duration of the right ventricular MAP during spontaneous rhythm is associated with a prolongation as well as a diminished repolarization rate of phases 1–2 and 3

The causes of these variations in duration and configuration of the MAP are by no means obvious. Apart from the rate dependence already discussed, the configuration of a ventricular AP from a specific species is influenced also by factors such as ionic composition of the surrounding fluid (30) presence of digitalis (23) or quinidine (7), and age of the cell (19)

The increased rapidity of the repolarization during phase 3 associated with higher spontaneous heart rate and shorter duration of the MAP might at least partly be explained by the observation that epinephrine increases the repolarization rate of phase 3 of APs from dog papillary muscles (10) Ionic composition of the blood with respect to Na, K, Ca, Cl, phosphate and bicarbonate is normal, but may still of course be a factor partly responsible for the observed variations of the duration of the MAP In few patients calcium and phosphate were not measured, but there are no grounds for suspecting any abnormalities of these ions in the patients.

The rapid upstroke of the MAP represents the time of excitation of the myocardium in the immediate vicinity of the suction electrode (11). Thus the various Q-phase-0 intervals in the different recordings are helpful in estimating the position of the suction site of the catheter The excitation of the right ventricular endocardial surface has been shown to start 5 to 10 msec and to be completed 60–70 msec after the onset of

the QRS complex (6) The present investigation has a similar range (0–67 msec) in all cases with normal activation of the right ventricle. In the two cases with RBBB there is a late activation of the part of the ventricle explored by the suction-electrode-catheter as might be expected.

The amplitude of the right ventricular MAP is higher than that of the right atrial MAP (25). As discussed elsewhere this is probably due to different thickness of the subendocardial connective tissue layers (25)

VCG changes in right ventricular hypertrophy due to pulmonary stenosis are well correlated to the haemodynamic severity of the disease (14). These VCG abnormalities are apparently due to an increased number of muscle cells (14) The pure effect of right or left ventricular hypertrophy on the configuration of an AP has not been found to be reported in the literature. The AP of a cardiac myofibril from a patient with hypertrophic obstructive cardiomyopathy has revealed a prolongation of the repolarization time (3) However the hypertrophy in obstructive cardiomyopathy is different from the general left or right ventricular hypertrophy in valvular diseases. In the few patients with pronounced elevation of the pulmonary artery or right ventricular systolic pressure, there are no systematic changes in the configuration of the right ven MAP However these few observations be considered to be of general validity

Increased right atrial pressure might be associated with a dilatation of the heart and stretching of the muscle fibres. Stretching of a ventricular muscle specimen may cause after potentials in the AP (5) However it is unlikely that tension in the intact in situ heart often reaches this level" (10) and no afterpotentials of the type described have been noticed in the patients investigated.

Pathologically increased intracardiac pressures might also lead to endocardial thickening with a drop of the MAP amplitude (25) This has not been observed in the present investigation, suggesting the absence of general right ventricular endocardial thickening in these cases.

The rate dependence of the duration of a entricular AP is a well-known phenomenon (8, 9 12, 22, 32, 33) The relationship has been reported to be linear for the human ventricular muscle (8), though not below the rate of 48/min (33) Also the relationship between the QT interval and the heart rate has a good linear correlation in experiments using right ventricular pacing of the human heart with frequencies between 35 and 135/min (31) In these experiments the logarithmic, square root and cube root functions were not superior in fitness compared to the linear one. The present investigation has revealed that the same holds true for the relationship between the duration of the right ventricular MAP and the cycle length when this is varied by atrial pacing and ranges between 1 151 and 382 msec, corresponding to heart rates of 52 and 157 beats/min.

The heart rate changes induced by atrial pacing are not accompanied by the general haemodynamic adaptation which is seen when the heart rate increases due to physical exercise (28) Neither does the coronary blood flow reach the same high level as when the heart rate increase has been caused by physical exercise (13) The lack of sympathetic discharge associated with the tachycardia produced by atrial pacing might also mean that the electrophysiological phenomena of the heart cells are different during this kind of heart rate increase. The prolongation of the P-Q time or development of A V block II during atrial pacing at high rates (21) was constantly observed in the patients investigated. This prolongation of the P-Q time might be caused by the lack of increased sympathetic nervous activity upon the atrio-ventricular node (21). The change in configuration of the right ventricular MAP during atrial pacing is in agreement with the observations in studies on the rate dependence of ventricular muscle AP from tissue specimens (8, 12, 33) These studies have revealed that, during increasing regular rates, the ventricular AP shortens due to a shortening of phase 2. Sympathomimetic amines cause an increased repolarization rate during phase 3 (10) This was not observed in the present material during pacing-induced heart rate increase. It is possible that the same heart rate increase induced by sympathetic nervous discharge might have been associated with an increase in RRR of phase 3 of the MAP

ACKNOWLEDGEMENT

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RIGHT VENTRICULAR MONOPHASIC ACTION POTENTIALS IN MAN

Effect of Abrupt Changes of Cycle Length and of Atrial Fibrillation

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Abstract. Right ventricular monophasic action potential (MAP) has been recorded during abrupt changes of cycle length and during atrial fibrillation in patients undergoing heart catheterization. The abrupt cycle length changes were initiated by sudden changes of stimulation rate using right atrial pacing. A sudden prolonged cycle is terminated by MAP with increased duration and decreased repolarization rate of phase 3. An acute shortening of the cycle is followed by MAP with decreased duration. The repolarization rate of phase 3 of such MAP is not generally increased as expected from in vitro studies. In atrial fibrillation short cycle is followed by MAP with short duration and rapid repolarization rate of phase 3, whilst long cycle is terminated by MAP with long duration and slower phase 3 repolarization. The similarity between the adaptation of the MAP configuration after abrupt changes of cycle length and during atrial fibrillation suggests that rate-dependent adaptation of the contractile force—known from earlier in vitro studies—might also occur during atrial fibrillation in man.

Abrupt alterations of cycle length are associated with characteristic changes in the configuration of the action potential (AP) of a heart muscle strip (3, 4, 5, 6, 8, 9, 10, 14, 22). Edmands et al. reported that the mammalian ventricular AP terminating a relatively short cycle has an unchanged duration but a prolonged phase 2 and a more precipitous phase 3 compared with the preceding AP (3, 4, 5, 8, 10). They also reported that a relative prolongation of the cycle length is succeeded by an AP with shortened phase 2 and prolonged phase 3 which exhibits a slower repolarization resulting in an unchanged duration of the ventricular AP. These changes in cycle length and AP configuration are combined with an adaptation of the contractile force of the muscle (4, 10, 11) and have been suggested to be due to changes in the transmembrane potassium flux or permeability (3, 4, 10). An inverse relationship between the duration of phase 2 of the AP and the contractile force has been suggested (4, 10).

All the above-mentioned studies have been performed on excised muscle fibres and to our knowledge no similar study has been performed on the intact human heart. Though difficulties exist in recording the AP of the heart even during open chest surgery, the monophasic action potential (MAP) can easily be recorded from the intact human heart (20). The similarity between a MAP and an AP during the repolarization course (13) permits evaluation of proportional changes in the AP configuration from MAP recordings. This has prompted us to study the adaptation of the right ventricular MAP after abrupt alterations of the cycle length during regular rhythm. In addition, we have studied the right ventricular MAP during unpredictable sudden changes in the cycle length initiated by atrial fibrillation.

METHODS AND MATERIAL

The MAP recording method used has been described elsewhere (20), as have the criteria for an acceptable MAP recording (20) and the method for analysis of an individual ventricular MAP (18).

Abrupt cycle length changes

Fourteen patients in sinus rhythm undergoing routine diagnostic heart catheterization have been investigated with right ventricular MAP recording. The patients are identical to those investigated with atrial pacing in an earlier report (18). The cycle length could be controlled by right atrial pacing, using bipolar pacemaker catheter (USCI 5652), introduced percutaneously from brachial vein, and an external pacemaker (Elema 138 or American Optical 10970R). The pacing rates have been 73, 86, 100 and 150 impulses/min. Right ventricular MAP has been

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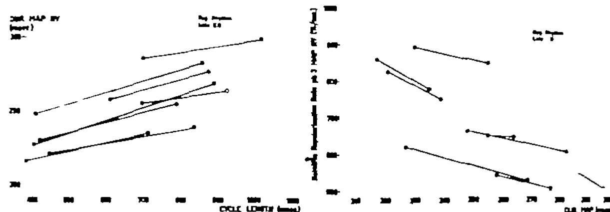


Fig. 5. Adaptation of duration (left figure) and RRR of phase 3 (right figure) of MAPs terminating a long cycle during regular rhythm. There is statistically significant increase in the duration of the MAP of the late extra beat (EB). Phase 3 repolarization of the late MAP is also statistically significantly decreased.

Figs. 1 and 4. The prolongation of the cycle length was on average 352.8 msec (69.1%). The cycle length prolongation was constantly followed by a prolongation of the duration of the right ventricular MAP (Fig. 5) also being significant ($p < 0.001$). The RRR of phase 3 of the unexpected late MAP decreased in all 8 recordings as seen in Fig. 5 ($p < 0.005$). The most pronounced changes in duration or RRR of phase 3 are observed in the recordings with the most pronounced prolongation of the cycle length.

Phase 1–2 of the MAP could be identified in recordings before as well as after the sudden cycle length prolongation. In these recordings the prolongation of the MAP was constantly accompanied by a prolongation of phase 1–2 and in two cases also of phase 3. The RRR of phase 1–2 decreased in two and increased in one case.

Atrial fibrillation

Thirty-one recordings, each including 6–22 consecutive right ventricular MAPs, were obtained from the 11 patients. An example of a MAP recording during atrial fibrillation is given in Fig. 6. There is in all recordings a relationship between the cycle length and the duration of the MAP terminating this cycle. The duration of the MAP is increased with increasing preceding cycle length (Fig. 7). The regression coefficient is less than 0.5 in only one of these recordings. On average the prolongation of the MAP duration is, to an extent of $^2/_3$, due to a prolongation of the duration of phase 1–2 and, to an extent of $^1/_3$, of phase 3. The RRR of phase 3 of the right ventricular MAP mostly increases with decreasing duration of the same MAP during the consecutive MAP recordings (Fig. 8). RRR of phase 1–2 could



Fig. 6. Example of right ventricular MAP and electrogram (RVE) recording during atrial fibrillation (case 41). For further information see text.

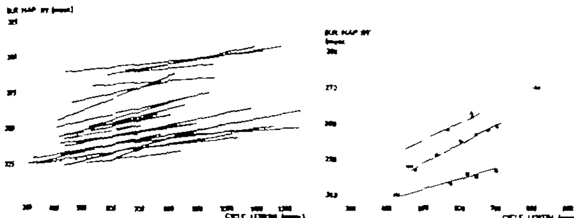


Fig. 7. Adaptation of duration of right ventricular MAP during atrial fibrillation. The right figure presents the data from the analyses of individual representative recordings in three patients (recordings 3 *a*, 26 and 27 *e*) as well as the mean square fitted regression lines in these recordings. The left figure presents the regression lines of all 31 recordings. —— > 0.50 < 0.50.

be identified in 19 of the 31 recordings. In 17 of them there was an increasing RRR of phase 1–2 with decreasing MAP duration and in 8 of them with a regression coefficient more than 0.5

DISCUSSION

The configuration of the right ventricular MAP in this study is in agreement with earlier observations on right ventricular MAP during regular rhythm (18) and the AP from excised human ventricular muscle (23). As the MAP is a reliable approximation of the repolarization course of an AP from a cell in the close vicinity of the MAP recording point (13) the various configurations of the MAPs most probably represent real changes in configuration of the AP

Hoffman and Suckling investigated the effect of different rates of stimulation on the AP from excised dog papillary muscle (14). They reported that the AP terminating a long period of quiescence had a long duration and that during the following regular rhythm the successive APs exhibited alternation of duration. They also reported that the plateau of the early interpolated AP was slightly prolonged and the slope of the final phase of recovery increased". This characteristic adaptation of the AP elicited by an early interpolated stimulus is also apparent in a figure in a later report by Hoffman et al. (11). Analysis of the early interpolated APs in these reports by the method used in the present investigation (18) reveals a decreased duration of the AP and an increased repolarization rate of phase 3.

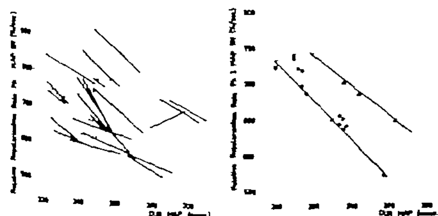


Fig. 8. Adaptation of RRR of phase 3 of right ventricular MAP during atrial fibrillation. The right figure presents the data from the analyses of individual representative recordings in three patients (recordings 3 *a*, 26 and 77) as well as the mean square fitted regression lines in these recordings. The left figure presents the regression lines of all 31 recordings. —— 0.50 < 0.50.

Edmands et al. reported in a series of papers (3 4 5 8 9 10) that the AP of excised dog and human ventricular muscle fibres exhibits no change in total duration after abrupt alterations of cycle length. They measured the total duration of the AP from the onset of phase 0 to the time when phase 3 has reached the baseline (4) The duration of phase 2 was arbitrarily defined as the time from the onset of phase 0 to the time of 20% repolarization (4) Thus it is not possible to compare directly the results from the present investigation with their reported results. Analysis of their reported original figures of right ventricular APs (3 4 5 9) by the method used in this report, however reveals that the AP of an early extra beat has a shortened duration with steeper phase 3 When phase 1–2 has been possible to define it appears that this is also abbreviated in the AP of an early extra beat. Similarly the AP of an unexpected late beat has in some reports a prolonged duration (3 5) with decreased repolarization rate of phase 3 but in other reports probably an unchanged duration in spite of a slower phase 3 repolarization (9 10) These contradictory findings might be explained by the fact that the basic stimulating frequency has been about 60/min, below which the rate-dependent changes of the dog ventricular APs are no more obvious during regular rhythm (14) The retouched figures reported (4 10) mostly fail to demonstrate the facts pointed out, and the authors have above all devoted their attention to the APs and potentiation of contractions of the postextrasystolic beats.

Surawicz et al. reported that the duration of the ventricular AP of isolated perfused rabbit heart became shorter with increasing prematurity of the beat (22) It is also evident from their reported figures that the AP of the early interpolated beat has an increased repolarization rate during phase 3 This characteristic configuration of the early interpolated AP is also evident in a report by Moore et al. (16) The duration of the premature AP may however also depend upon the level of the membrane potential at the time of initiation of depolarization (6) When the resting membrane potential became less negative the effect of the prematurity of the interpolated stimulus on the duration of the AP decreased progressively (6) This mechanism might be of importance in the intact heart when a new depolarization is initiated before total repolarization has been reached, e.g. during early extra beats or rapid tachycardia. No MAPs of this type are recorded in the present investigation.

The change of configuration of the right ventricular MAP terminating a prolonged cycle in otherwise regular rhythm in the present study is in agreement with that of the mammalian ventricular AP during comparable rates and cycle length changes.

The adaptation of the right ventricular MAP duration after abrupt shortening of cycle length in the present study is in agreement with that found in the AP studies cited above. The failure to identify a statistically significant increased repolarization rate during phase 3 of the early extra MAP might be due to many causes. The specific conditions during in vitro studies might be contributory Thus the normal neurohumoral influence upon the cell membrane is lacking in the in vitro experiments. Sympathomimetic amines tend to enhance the repolarization rate during phase 3 (12) The ionic composition of the perfusion fluid used differs from that of blood. The potassium concentration is, for instance, rather low in Tyrode's solution. A low extracellular potassium concentration increases the transmembrane potential difference during phase 4 and prolongs the AP duration (12, 16) On the other hand, the MAP recordings are also associated with factors which might explain the lack of agreement between the results of the present investigation and the in vitro studies. The hearts from which the MAP recordings have been made have all been diseased in various ways and have been affected by different drugs. Digitalis shortens the duration of a ventricular muscle AP (17) Diuretics are known to cause an intracellular potassium depletion (2) The adaptation of the MAP has been studied after five heart cycles differing less than $\pm 10\%$ of their mean duration. This period is so short that the refractory period of the ventricles has not reached steady state Janse reported that the refractory period of the dog myocardium did not reach steady state until after several hundred beats following a sudden increase of heart rate (15) He also reported that the refractory period of the purkinje fibres became shorter than that of the myocardium during an early extra beat, quite opposite to the condition during regular rhythm However the

most probable reasons for the failure to demonstrate increased RRR of phase 3 of the early MAPs in the present material are that the abbreviation of cycle length has been much less pronounced than in the cited studies with microelectrode techniques and that the calculation of RRR of phase 3 in olves some uncertainty (19)

No special attention has been paid in this study to analysis of the well known T wa e changes after cycle length changes (1) However it is evident from the figures that the various configurations of the MAPs are associated with dif ferent changes in the configuration of the T waves.

Neither has the eventual augmentation or re duction of contraction in connection with the cycle length alteration been studied here. The postextrasystolic potentiation of contraction is not merely of theoretical interest, as this mechanism can be used clinically Paired stimulation of the heart has thus been applied in cases with heart failure to improve contractile performance (7). Unfortunately the drawbacks of this method have been so pronounced that it can only be recommended for highly selected cases.

Atrial fibrillation is associated with largely unpredictable variations in cycle length. The re cordings during trial fibrillation reveal a uniform pattern of adaptation of the right ventricular MAP A short cycle is terminated by a MAP with the main characteristics of that of an early beat interpolated in regular rhythm. Similarly a long cycle is terminated by a MAP of the type following a long cycle during regular rhythm. All patients except one received digitalis. The findings in this patient do not deviate from those of the digitalis-treated patients. *In addition* we have been able to confirm this pattern of adaptation of right ventricular MAP in one patient with sinus rhythm and free from digitalis. This patient (case 7) has been presented in an earlier report on right ventricular MAP recordings during regular rhythm (18) He was investigated with right ventricular MAP recording during right atrial pacing. The atrioventricular conduction was ir regular resulting in irregular xcitation times of the ventricles.

The present study has revealed that a cycle length alteration is associated with typical changes in the configuration of the right ventricular MAP irrespective of the reasons provoking the cycle length alteration, e.g. artificially by atrial pacing or pathophysiologically by atrial fibrillation. In vitro studies have revealed that cycle length alterations are accompanied by an adaptation of the contractile force as well as of the AP configuration. Thus atrial fibrillation is presumably accompanied also by a beat-to-beat adaptation of the ventricular contractile force depending upon the alterations of cycle length. This mechanism would thus act on the ventricles during atrial fibrillation independently of and simultaneously with the Starling mechanism.

ACKNOWLEDGEMENT

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Table I *Data concerning the subjects investigated in a series of medico-legal autopsies in Addis Ababa*

Figures in parentheses denote females

Cause of traumatic death	No of subjects in different age groups (y)				
	< 20	20–39	40–59	≥ 60	Total no
Road accidents	7 (2)	32 (1)	19 (3)	3 (2)	61 (8)
Criminal assault	2 (0)	30 (3)	11 (0)	2 (0)	45 (3)
Suicide	1 (0)	2 (0)	(0)	0	5 (0)
Electric shock	1 (0)	3 (1)	0	0	4 (1)
Hanging	1 (0)	10 (0)	5 (0)	0	16 (0)
Suffocation, drowning	2 (1)	6 (0)	4 (0)	0	12 (1)
Other accidents	3 (0)	6 (1)	2 (0)	0	11 (1)
Total	17 (3)	89 (6)	43 (3)	5 (2)	161 (14)



Fig. 1 Schistosomiasis in a man, aged 30, killed by criminal assault. Egg-shells of Schistosoma are seen in the centre of a parasitic granuloma in the liver Methenamine Silver Method, 325

with necrosis, and this was probably a case of miliary tuberculosis, though no acid fast rods were seen in the sections. In two of the granulomatous cases eggs of Schistosoma were revealed (Fig. 2) In the other 12 cases the cause or causes of the granulomata could not be determined morphologically

Pronounced and widely disseminated *fatty changes* in the liver cells were found in seven cases, i.e. 4% of the series. The occurrence of occasional fat-loaded cells in the liver was not considered a significant pathological finding and is therefore not included under this heading. In one of the seven cases the fatty changes were accompanied by granulomata, probably tuberculotic, and cirrhosis, and in another three cases of fatty changes haemosiderosis was present in addition. In one person with fatty changes there was also pronounced venous congestion

Portal fibrosis was found in 10 cases, i.e. 6.2% of the series, and *cirrhosis* in a further 6 cases, i.e. 3.7%. The term portal fibrosis means here a moderate increase of the fibrous tissue in the

II. *Pathological changes in the livers of the subjects investigated*

Figures in parentheses denote females

Pathological changes		No. of subjects (= 38)	% of the whole series (n = 161)
I	Granulomatous	15 (1)	9.3
II	Tuberculotic	1 (0)	0.6
III	Parasitic	2 (0)	1.2
IV	Unknown etiology	12 (1)	7.5
V	Fatty changes	7 (0)	4.3
VI	Portal fibrosis	10 (1)	6.2
VII	Cirrhosis	6 (1)	3.7
VIII	Haemosiderosis	4 (0)	2.5
IX	Severe venous congestion	1 (0)	0.6
X	Probably septicaemia	1 (0)	0.6
Combinations of lesions listed above			
II + V + VII		1 (0)	
III + VI		1 (0)	
V + VIII		3 (0)	
V + IX		1 (0)	
VII + VIII		1 (0)	

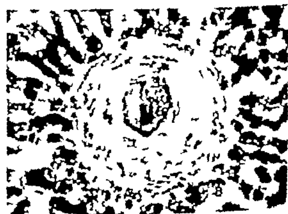


Fig. 2. Schistosomiasis in another man of the same age also killed by homicide. Egg of Schistosoma in the centre of granuloma in the liver H & E. 325

portal areas without derangement of the lobular pattern of the parenchyma. By cirrhosis is meant pronounced fibrosis with derangement of the normal lobular pattern and signs of nodular regeneration of liver cells. It was difficult to determine the exact type of cirrhosis, but most cases seemed to belong to the "portal" or "nutritional" type, i.e. the Laennec type. In only one case of cirrhosis were there in addition fatty changes of the liver cells. In one case there was an additional haemosiderosis with abundance of haemosiderin pigment in the liver cells, in the Kupffer cells and in the macrophages in the portal areas. A few more cases with cirrhosis and fibrosis showed only sparse haemosiderin pigment in the sections, which was not considered significantly pathological and therefore not recorded as haemosiderosis. In one case the cirrhosis was combined with Schistosomiasis.

Haemosiderosis with abundance of haemosiderin pigment in the liver cells, in the Kupffer cells and in macrophages in the portal areas was revealed in four cases; in one of them it was combined with cirrhosis and in three with pronounced fatty changes.

Septicaemia was probably the cause of multiple small abscesses in the liver of an infant who died t the age of three months of a cranial trauma.

There were no morphological signs of virus hepatitis in any of the cases in this series, nor was any tumour growth in the liver observed.

DISCUSSION

Are the liver lesions found in this autopsy series representative of the whole population of adult men in the Addis Ababa region during the two periods in question? It is difficult to answer this question. As the persons in our series were killed by traumata and not by the liver lesions, it may be argued that with respect to these liver lesions they were selected by chance. From this standpoint the frequency of each of the li er lesions found should tally well with the prevalence of this lesion in the whole male population in the area. However this hypothesis is valid only if there are no indirect connections between the traumatic death and the liver changes. Theoretically there may be such connections, e.g. between alcoholism, fatty changes, and cirrhosis, on the one hand, and road accidents and suicide on the other The ery sparse records of the dead persons permit no pertinent conclusions in this respect.

The percentages of portal fibrosis and cirrhosis in this series were 6.2 and 3 7 respectively Bothwell and Bradlow (1) on examining an autopsy series of 147 traumatic deaths among Bantus in South Africa, found portal fibrosis in 15.6% and cirrhosis in 1 4% The difference between the two series is not significant ($p > 0.05$) The reason for the apparently higher percentage of portal fibrosis in the series of Bothwell and Bradlow may be the inclusion of also slight degrees of deviation from the normal. In our experience it is hard to distinguish between the normal amount of fibrous tissue in the portal area and a slight pathological increase of fibrous tissue and consequently cases with a slight degree of portal fibrosis were not included.

A special feature of the diet in highland Ethiopia and also among the Bantu tribe is the very high content of iron. Bothwell and Bradlow (1) found in their series from South Africa some degree of siderosis in the liver in 89% of the cases, and in 19% the siderosis was severe with an iron content exceeding 1 000 mg/100 g dry weight. Hofvander (3) however found only a slight, non-significant increase of non-hemin iron in the livers of Ethiopian males, compared with the iron content in liver biopsies from Swedish control males examined by Weinfeld et al. (8) and he concluded that only a small part of the iron in the Ethiopian food is absorbed. This appears to be supported by the finding of only four cases, 2.5% of severe siderosis (haemosiderosis) in our series. Bothwell and Bradlow found a close correlation between siderosis, on the one hand, and fibrosis and cirrhosis, on the other exclusively in cases of a high iron content in the liver tissue, exceeding % of the dry weight. Thus, it is unlikely that the small amount of haemosiderin found in some of the 13 unexplained cases of portal fibrosis and cirrhosis in our series was the cause of these lesions.

In only three of the 15 cases of granulomatosis was a possible cause of the granulomata revealed (in two cases Schistosomiasis and in one case tuberculosis) From a review of the literature and a series of their own, Guickian and Perry (2) found such granulomata in 3–10% of livers

examined by means of biopsies and autopsies. However these figures concern hospitalized subjects. In Texas the same authors carried out a combined clinical, microbiological and histological investigation of 63 hospitalized patients with granulomatosis of the liver and found tuberculosis in 54% of the series. Tuberculosis is rather common in the Addis Ababa region (5) It is feasible that there were still more cases of tuberculosis among the 15 cases of granulomatosis in addition to the one mentioned above, but this is impossible to prove. It is obvious that the occurrence of tuberculosis in the liver in our series says nothing about the real frequency of the disease among the subjects, as tuberculosis in the liver only reflects the military type of the disease. It is possible that in some cases the granulomatosis might have been produced by infestation by amoebae, which can provoke this kind of lesion (6)

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DIFFERENT SERUM INSULIN PATTERNS IN PORTAL, HEPATIC AND PERIPHERAL VENOUS BLOOD IN TWO CASES OF INSULOMA

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Abstract. Insulin secretion has been studied in two cases of insuloma. In one of them neither fasting hyperinsulinemia nor excessive insulin release in response to glucose ingestion or tolbutamide administration could be demonstrated. This patient belonged to a family in which pronounced insulin responsiveness was observed. In the other case, with hypoglycemic attacks and hyperinsulinemia following β-cell stimulation, a higher insulin level was observed in hepatic venous blood than in portal blood, indicating extrapancreatic insulin-producing tissue.

It is generally accepted that the hypoglycemia connected with insulomas is due to hyperinsulinemia. However in some cases neither fasting hyperinsulinemia nor exaggerated insulin responses to tolbutamide, leucine or glucose are demonstrable (6). In the present investigation immunoreactive insulin (IRI) has been assayed in portal, hepatic and peripheral venous blood in two cases of insuloma in order to get a more complete picture of the insulin release in these cases.

CASE REPORTS

Case 1

A woman, 54 years old and non-obese when examined. She always felt restless and irritated in the mornings if she did not have her regular meals. No such symptoms occurred during the rest of the day. A brother, a sister and a niece had similar complaints, and the mother had manifest diabetes. During the three years preceding admission to hospital the symptoms changed, and she had numerous short attacks of unconsciousness in the mornings before breakfast. During such an attack hypoglycemia was found, and she was therefore admitted to hospital for further examination.

Case 2

A woman, aged 60, of normal weight and without family history of diabetes. She complained of frequent short attacks of unconsciousness since more than twenty years. These attacks occurred in the mornings a few hours after breakfast. During the last year the attacks occurred more frequently and therefore she was admitted to hospital for further examination. Fasting blood glucose concentrations in peripheral venous blood were at that time 76–116 mg/100 ml. As laboratory results indicated reactive hypoglycemia, a low carbohydrate diet was instituted, which resulted in a decrease in the hypoglycemic symptoms for some months. Because of a long attack of unconsciousness she was, however, admitted to hospital again.

METHODS

Blood glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi Reagents, Sweden).

Immunoreactive insulin (IRI) was assayed by a double antibody procedure essentially as described by Soeldner and Slone (12). Pork insulin (10× crystallized) was used for immunization. All insulin concentrations were determined by reference to a standard of 2× crystallized human insulin (obtained from Dr J. Schlichtkrull, Novo Research Institute, Copenhagen). *Normal fasting IRI* values in this laboratory are below 30 μU/ml (8).

Oral glucose loading (100 g glucose) was performed after an overnight fast.

Under fasting conditions 1 g of *tolbutamide* (20 ml 5% Rastinon, Hoechst) was injected into an antecubital vein over a period of 2 min. The midpoint of the injection was taken as zero time. Blood samples were drawn from the antecubital vein.

Catheterization. Pre-operative catheterization of the portal vein was performed by a transumbilic catheterization technique (5, 16) in both cases. Postoperatively a transhepatic catheterization technique (15) was used in case 2. The hepatic vein was catheterized through the femoral vein. The position of each catheter was checked by X-ray examination before the various tests.

Both patients were given a regular hospital diet before the test procedures.

examined by means of biopsies and autopsies. However these figures concern hospitalized subjects. In Texas the same authors carried out a combined clinical, microbiological and histological investigation of 63 hospitalized patients with granulomatosis of the liver and found tuberculosis in 54% of the series. Tuberculosis is rather common in the Addis Ababa region (5). It is feasible that there were still more cases of tuberculosis among the 15 cases of granulomatosis in addition to the one mentioned above, but this is impossible to prove. It is obvious that the occurrence of tuberculosis in the liver in our series says nothing about the real frequency of the disease among the subjects, as tuberculosis in the liver only reflects the miliary type of the disease. It is possible that in some cases the granulomatosis might have been produced by infestation by amoebae, which can provoke this kind of lesion (6)

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100 ml (mean 49.4 mg/100 ml) and the corresponding fasting IRI values from 1–16 μU/ml (mean 10.4 μU/ml). An oral glucose load resulted in a low glucose curve in peripheral venous blood and an IRI maximum of 1 020 μU/ml after 45 min (Fig. 2). After catheterization an additional oral glucose load revealed a considerably higher IRI maximum in the hepatic than in the portal and peripheral venous blood (Fig. 5). Since the load was performed in the afternoon this test cannot be compared with an ordinary oral glucose load after an overnight fast.

Celiaco-mesentericography revealed a tumor in the head of the pancreas but no metastasis was found. At operation an insuloma in the head of the pancreas (the diameter of the tumor was 2.5 cm) and a small insuloma in a lymph node behind the head of the pancreas were extirpated. Both insulomas were histologically confirmed, but whether the tumor in the lymph node was of metastatic or ectopic origin could not be settled. No further hypoglycemic symptoms have appeared after the operation.

Post-operative investigations in case 2

In Fig. 2 the result of an ordinary oral glucose load is demonstrated. Fig. 6 shows the result of an additional oral glucose load followed by an i. tolbutamide administration and an i.v. glucose

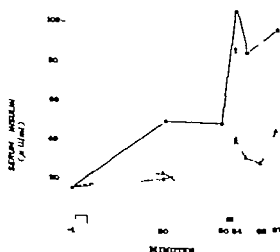


Fig. 3. Case 1. Immunoreactive insulin in portal (O—O), hepatic (● ●) and peripheral venous blood (▲ ▲) in response to pre-operative oral glucose load (□ – glucose given in 0–4 min interval) followed by an i. tolbutamide injection (■ – injection performed between 51–53 min).

Table I. *Peripheral venous insulin (I μU/ml) and glucose (G mg/100 ml) in response to i.v. tolbutamide administration pre and post-operatively*

	Tolbutamide administration						
	−2	2′	10′	20	60	150	180
Pre-operative							
I (case 1)[a]	20	42	48	—	—	—	—
I (case 2)[a]	12	812	914	—	—	—	—
G (case 1)[a]	16	—	14	—	—	—	—
G (case 2)	42	—	40	—	—	—	—
Post-operative							
I (case 1)	8	30	12	—	10	6	8
I (case 2)[b]	4	570	370	—	—	—	—
G (case 1)	59	—	45	31	18	38	47
G (case 2)[b]	60	57	40	—	—	—	—

[a] Discontinued after 10 min because of hypoglycemic symptoms.
[b] Discontinued after 10 min because the patient was undergoing discomfort.

infusion after catheterization of the portal and hepatic veins.

In three relatives of case 1 oral glucose loads were performed. The results are given in Fig. 4. The corresponding values obtained in a control group (8) are plotted for comparison.

DISCUSSION

In case 1 fasting IRI as well as insulin response to glucose and tolbutamide fell within the normal range in peripheral venous blood. It is noticeable, however, that IRI under fasting conditions was often in the upper half of the normal range when blood glucose concentration was less than 20 mg/100 ml, indicating relative hyperinsulinemia described in connection with insuloma (7). Considering that several blood samples were drawn at different times in the mornings, all showing normal IRI values, it seems unlikely that a transient insulin peak preceding the symptoms of hypoglycemia could have been missed.

Samols and Marks (11) reported an elevated level of insulin in portal blood associated with a normal IRI level in peripheral venous blood in a case of insuloma. This was thought to be due to removal of insulin by the liver. In case 1, however, high hepatic retention of insulin as a factor contributing to the normal peripheral IRI values could not be demonstrated (Fig. 3), which

Table I *Data concerning the patient and his father*

Examined in	Patient 1967	Patient 1968	Patient 1969	Father 1967
Age (y.)	15	16	17	45
Hb (g/100 ml)	14.0	13.4	14.1	14.0
RBC (10^6/mm³)	4.8	4.9	4.5	4.8
Hct ( )	46	49		45
MCV (μ³)	98	100		95
MCHC (g 100 ml)	28	28		29
Reticulocytes (10^3/mm³)	140	278	120	112
WBC (10^3/mm³)	7.2	6.2	7.8	7.6
Platelets (10^3/mm³)	140	84	120	
Serum bilirubin (mg 100 ml)	6.9	10.0	5.5	3.1

Mostly unconjugated.

and had a dark urine died in his fifties in a concentration camp The patient father had jaundice, dark urine and enlargement of the liver and the spleen. On the mother's side no member had had such symptoms. In 1955 he was hospitalized because of measles. H had no jaundice or enlargement of liver or spleen. Hb 13.3 mg/100 ml, RBC 4.3 mill., reticulocytes 4.9% (e. about 200 000 mm³). WBC, differential count and osmotic fragility were normal. Serum bilirubin was 1.2 1.8 mg/100 ml. Since then he had been healthy The hyperbilirubinaemia later became clinically apparent and his jaundice fluctuated irregularly The urine was redbrown but the stools were of normal colour Some attacks of epigastric pain were thought to be precipitated by gallstones. He came to J. Waldenström, Malmö, to get advice concerning splenectomy He was mentally and physically developed young man with jaundice and with palpable four fingerbreadths below the costal arch. ray revealed gallstone of 1 cm diameter

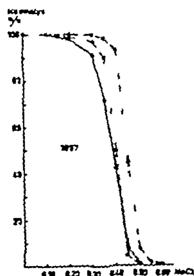


Fig. 1. Osmotic fragility of the patient. Normal range ----.

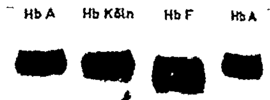


Fig. 2. Agar gel electrophoresis at pH 7.0 of haemoglobin A and haemoglobin from the patient.

RESULTS

Haematological data are given in Table I which includes data regarding the father who was not re-examined as he accompanied the patient only on the first trip to Malmö. The shortened life span of th. red corpuscles was compensated by an intense production reflected as a marked reticulocytosis (upper border of normal range 50 000 mm³)

There was anisocytosis and some crenelated cells but no spherocytosis. No Heinz bodies were seen in fresh smears of peripheral blood, but after incubation at 37 C for 48 h (3) inclusion bodies were seen in almost all the cells.

Signs connected with hyperhaemolysis, beside reticulocytosis and an increased concentration of unconjugated serum bilirubin (Table I), were a serum haptoglobin concentration of 2 mg/100 ml (lower border of normal range 70) and an increased endogenous CO production (COHb 1.3 Vol.% > 0.75 speaks strongly in fa our of hyperhaemolysis) The osmotic fragility was somewhat reduced (Fig. 1). The concentration of glucose-6-phosphate dehydrogenase and pyruvic kinase was increased, probably due to the high number of young cells.

Haemoglobin electrophoresis in agar gel at pH 7.0 revealed a fraction with a mobility slower than Hb-A (Fig. 2).

Heating a buffered haemolysate at +50 C (3) gave a whitish yellow precipitate. After 3 h 15 of the haemoglobin had precipitated.

The fingerprint of the purified and aminoethylated abnormal haemoglobin is shown in Fig. 3 At pH 6.4 βTpXI was mi ed up with other peptides but the Köln-βTpXI could be identified

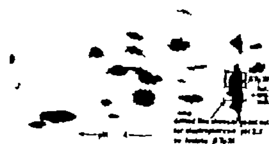


Fig. 3. Fingerprint of purified and amino-ethylated haemoglobin.

by giving three staining reactions, one each for histidine, arginine and methionine. This area was cut out and re run at pH 3.5 The histidine positive peptide could then be separated from the others and was further analysed (Table II) There was a fair amount of valine present, which shows the difficulty of separating Hb-Köln from Hb-A to such an extent that one gets the pure methionine peptide. The very presence of methionine, however is enough to indicate that this is Hb-Köln.

The oxygen affinity of heparinized whole blood was determined and a dissociation curve was constructed, showing an increased oxygen affinity (Fig. 4). This part of the investigation was performed at the Department of Clinical Physiology

The dark urine contained no uroerythrin or free iron (Prussian blue)

Table II. *Analysis of the histidine positive peptide*

Amino acid	No. of residues Found	Expected
Aspartic acid	1.92	2
Threonine	0	0
Serine	0.34	0
Glutamic acid	1.19	1
Proline	0.96	1
Glycine	0.37	0
Alanine	0.31	0
Valine	0.60	1
Methionine	0.34	0
Isoleucine	0	0
Leucine	0.93	1
Tyrosine	0	0
Phenylalanine	0.99	1
Lysine	0	0
Histidine	1.27	1
Arginine	0.73	1

COMMENTS

The first report on Hb-Koln dealt with a family which had lived in the Rhine area for centuries. The subsequent families reported from Scotland and England were found to have a German ancestor in common. The Australian Hb-Köln families were of English-Scottish descent. The possibility that all these families are descendants of a single person with Hb-Koln cannot be excluded. It is well-known that the Jewish community in Poland has lived there for a very long time and has not had much immigration from outside. Therefore it seems probable that the mutation leading to Hb-Koln in our patient's family might be a new one and separate from that in the families mentioned above.

The question of immediate practical interest was splenectomy The postsplenectomy course in the few cases reported, who had been operated upon, do not give the impression of unequivocal therapeutic success (5 10) Our patient had no anaemia and, beside the gallstone, he had no trouble from his hyperhaemolysis. The attacks of biliary colic did not trouble him much, so we advised against splenectomy for the time being. His father who also had hyperhaemolysis, had not been much bothered by that condition.

The regenerating activity of the patient's bone marrow was extremely high. As there was no anaemia, it is probable that the increased oxygen affinity with hypothetical lowered delivery of oxygen of the blood was the stimulus to the bone marrow (1)

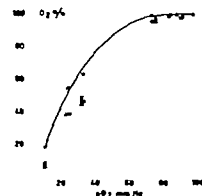


Fig. 4. Oxygen dissociation curve. ● the patient; symbols denote controls

There are few data on the life history of patients with Hb-Köln. Polish Jews have for centuries been exposed to extreme hardships. Unless the present condition is the outcome of a recent mutation, it seems that it does not affect health too seriously before the end of the period of fertility

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CALIBRATED HYPOXEMIA TEST IN NORMAL SUBJECTS AND CORONARY PATIENTS

I Hemodynamics and Acid-base Equilibrium in Hypoxemia of Short Duration

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Abstract. There is great need for calibrated test that can be used in coronary patients for diagnostic purposes and for evaluating the effect of drugs. A hypoxemia test with 7 / % oxygen in nitrogen on mouthpiece, with continuous registration of the oxygen saturation of the blood, has been evaluated in 13 patients with coronary disease and in 5 normal subjects. The test usually gives decline of the oxygen saturation of the blood to 70% or less, the level necessary to give maximal positive response. The dispersion of oxygen saturation is rather great. During hypoxemia of short duration the heart rate and the cardiac output will increase, but will never compensate for the decline of oxygen saturation. The change in acid base equilibrium is very slight. No significant change of BP occurred during hypoxemia. The degree of oxygen saturation will therefore give good indication of the coronary strain and may provide reproducible and calibrated test for use in coronary insufficiency

ECG examinations of coronary insufficiency are of great diagnostic importance in coronary diseases.

The most common of the many methods employed, the exercise test, has been standardized in different ways, the simplest being the old two-step test of Master (19).

In the hypoxemia test the subject respires air of varying oxygen content, as a rule not below 7 1/ % oxygen in nitrogen. The duration of the test has also been varied. Usually the test has been interrupted when angina pectoris or ECG changes have occurred, or when oxygen saturation of the blood has fallen to a ery low level. The test has generally lasted 10 min, no form of calibration being used.

Comparisons between exercise tests and the hypoxemia test have been made by several investigators (3 13 15). The difference in sensitivity was not found to be great In an earlier paper (13) it was found that it is necessary to reach an arterial oxygen saturation of at least 70% in order to obtain the maximal positive response.

There is a great need for a standardized and reproducible method which can be used as a measure of coronary insufficiency and, particularly for the evaluation of the effect of different drugs in coronary patients.

The purpose of the investigation presented here has been to examine the hypoxemia test with a view to ascertaining its value in the quantitative evaluation of coronary insufficiency

In the first part f the in estigation the hemodynamics and some biochemical changes in hypoxemia of short duration are studied to see whether parameters other than hypoxemia are of such importance that they may influence the result and represent an uncontrollable factor

Some compensatory changes of the hemodynamics occur in hypoxemia Vasodilation and tachycardia have been observed (11) also an immediate increase of cardiac output and coronary flow (9 12). Hultgren and Grover (14) found the same changes after an arrest of respiration of only 30–60 sec. A correlation between cardiac output and coronary flow (11) and a significant relationship between coronary flow the oxygen consumption of the myocardium and the mean aortic pressure ha e been demonstrated (1).

Kontos et al. (16) found in experiments with animals that 7 /₂% oxygen respiration induced an increase of cardiac output and heart rate if the respiration was allowed to increase spon-

Table I *The material and examinations done*

The f gures in parentheses represent women

	No of subjects	Cardiac output	Intra-arterial BP	Acid–base equilibrium
Ang. pect	13 (5)	6	2 (1)	3 (2)
Normal subjects	5	4	3	1

taneously The PCO_2 remained unchanged and mean arterial pressure was a little reduced.

Gelborn (10) observed in cats that hypoxemia of 60–90 sec duration was followed by a moder ate increase of BP and heart rate, later followed by a reduction.

In man Malmström (18) found that 9% ox ygen respiration was followed, in normal subjects, by a fall of systolic and diastolic BP If CO_2 was added no BP changes were observed.

The ECG changes are a result of an insufficient supply of oxygen to the myocardium.

The condition of the coronary vessels is of conclusive importance for the flow Levy et al. (17) are of the opinion that ECG changes are due partly to ischemia and partly to the reduced ox ygen tension. They as well as Burchell et al. (5) claim that ECG changes occur independently of the oxygen saturation of the blood. However in these experiments the oxygen saturation of the blood was moderate not below 70%

Case et al (6) found an increase of the K and lactate concentration in the coronary sinus and observed that ECG changes in coronary insufficiency occurred simultaneously with these biochemical processes. Cohen et al. (7) also demonstrated an increase in the lactate concentration in the coronary sinus in angina pectoris

The occurrence of angina pectoris indicates hemodynamic impairment. increased heart rate elevation of systolic BP and of left ventricular end diastolic and pulmonary capillary pressure (7 20 23) The last findings could not be observed by Cohen et al. (7), but their patients were in a less advanced stage of the disease Rosland (22) observed during spontaneous angina pectoris a considerable decrease of cardiac output.

The hemodynamics were normalized about 2 min after the end of exercise, the S-T depression persisted 2 min more (23)

In acute coronary insufficiency Parker et al. (21) found no rise of left ventricular end diastolic and pulmonar capillary pressure after administration of nitroglycerine. The cardiac index and stroke volume increased but less than in normal subjects. Catheterization studies of the coronary sinus have also been made in coronary patients (4 10) Brachfeld et al. (4) studied the effect of nitroglycerine. They present evidence that, although coronary dilatation indeed occurs, it appears to be secondary to changes in myocardial oxygen requirements. Hemodynamic observations revealed a decrease in pressure in both peripheral and pulmonary circuits. The exact mechanism of the clinical effect of nitroglycerine does not seem to have been fully explained.

MATERIAL AND METHODS

I all, 30 persons were examined. In only 18 patients were all registrations sufficiently successful to permit a complete evaluation. Thirteen patients (5 women) had angina pectoris, 6 in a light degree. The control material consisted of 5 non-cardiac patients.

All patients were examined in the morning fasting and after at least half-an-hour's rest. A polyethylene catheter was introduced by Seldinger's technique into the brachial artery and intravenously to the vena cava superior During the examination the patient respired air through a mouthpiece which was later connected to tubes conducting gas mixtures of 7 / % oxygen in nitrogen. The hypoxemia test lasted up to 10–12 min. The cardiac output was calculated after the injection of indigo cyanine with double determinations with about 1 min intervals. The acid-base equilibrium was examined by Astrup method.

Oxygen saturation and dye concentration ere recorded continuously on CMI Densox-densitometer ith a blood suction of 16 ml min. The blood was later reinfused. The curves and ECG (V_1–V_4–V_5–V_6) were registered on Lumitron recorder I the cases in which the cardiac output was examined the hypoxemia test as performed about 3 min before registration of O saturation. The capacity of the suction pump was only 100 ml, corresponding to 6 min registration. The examinations are shown in Table I.

Table II *Cardiac output in l/min (average values)*

The f gures in parentheses show the percental increase

	Air respiration	Hypoxemia test: Test 1	Hypoxemia test: Test 2
Angina pect.	5.28	5 59 (6)	6 61 (25)
Normal subjects	5 84	6 63 (13)	7.20 (24)

Table III. *Hypoxemia test in case 25*

The figures in parentheses show the percental deviation compared with air respiration. Transported oxyhemoglobin – the product of cardiac output and oxygenated blood in relative values

	Air respiration	After hypoxemia test Test 1	Test 2
O_2 saturation (%)	94	62 (36)	62
Cardiac output (l/min)	5 0	5 85 (17)	6 4 (43)
HR	61	78 (28)	80 (31)
Stroke volume (ml)	87	75 (14)	80 (–8)
AT (sec)	24	17	17
Transported oxyhemoglobin	100	74 (–26)	80 (20)

RESULTS

Oxygen saturation declined rapidly during the first minutes of the test, later slowly in average for all examinations from 95% to 79% after 3 min and to 72% after 7 min.

As shown in an earlier paper (13) the disper sion is great.

A single case reached 52% oxygen saturation after approximately 1 min respiration and was restless. The test was therefore discontinued. When the test was repeated, the saturation level fell to 47%. Tests where then discontinued.

In another case inhalation of $7\,^1/_2$% oxygen did not lower oxygen saturation to more than 80% in the first and 70% in the second test. No serious complaints were observed.

Heart rate increased opposite to the oxygen saturation. In a erage from 71 at full saturation to 85 after 7 min.

Cardiac output increased, which is shown in Table II.

Stroke volume declined. During air respiration the a erage alue was 80 ml, after the hypoxemia test 75 ml, a reduction of 6.3%.

The average circulation time (appearance time, AT) was reduced during hypo emia from 16 8 to 15.5 sec.

Table III shows the figures for case 25 45-year-old man with moderate angina pectoris It is seen that the increase in cardiac output can far from compensate for the reduced oxygen saturation in blood. The oxygen supply will therefore be considerably reduced.

Acid–base equilibrium

A very small individual variation was found The same trends were found in all patients The a erage values are shown in Table IV The highest pH value after the hypoxemia test was 7.52 with an oxygen saturation of 71 % after 8 min

The hypoxemia test gi es only small changes in acid–base equilibrium, corresponding to a very moderate respiratory alkalosis. The changes found are similar to those demonstrated by Barach et al (2), who were unable to show any difference between normal subjects and cardiac patients This ery moderate alkalosis cannot ha e any influence on the ECG

The intra-arterial BP changed ery little after the hypoxemia test The average values before hypoxemia were 133/74 after hypoxemia 130/67 mmHg. This moderate reduction is without any effect on the ECG or the hemodynamics

DISCUSSION

The hypoxemia test, with 7 / % oxygen, will gi e varying degrees of oxygen saturation in the blood. Usually 70% oxygen saturation or less will be reached in 5–6 min, without any risk for the patient Oxygen saturation must be continuously registered to gi calibration of the test.

The changes in BP and acid–base equilibrium during the hypoxemia will be ery moderate and will not influence the ECG or the hemodynamics.

Table IV *Acid–base equilibrium before and during hypoxemia (average value)*

	pH	pCO_2	BE (mEq)	Tot. CO_2 (mEq)	Stand. CO_2 (mEq)	O_2 saturation ( )
Before	7 41	41 4	+2.4	29 7	25.9	97
During	7 47	34.7	+2.9	26 6	26.4	68

The increased circulation during the hypoxemia test does not compensate for the reduced oxygen saturation. The oxygen saturation of the blood will therefore provide an effective and reproducible calibration test for coronary strain in patients with coronary insufficiency

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CALIBRATED HYPOXEMIA TEST IN NORMAL SUBJECTS AND CORONARY PATIENTS

II The Relation between ECG Changes and Oxygen Saturation of Blood

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Abstract. In 13 patients with angina pectoris and 5 normal subjects hypoxemia tests have been carried out, with continual registration of the arterial O_2 saturation and ECG. A gradual depression of the T wave was found during hypoxemia in most cases. There as no difference in the pattern found in normal subjects and coronary patients. The S-T depression during hypoxemia was not found in the normal subjects. In 6 patients with angina pectoris the S-T change occurred in the course of 3–4 min, at somewhat differing degrees of saturation, depending on the degree of angina pectoris. When the mask was removed, the arterial saturation rose rapidly in the course of approximately 30–40 sec. In most cases the ECG as normalized before saturation was complete. In few cases the ECG changes persisted for some minutes before full saturation occurred. On repeated tests in the same patient the S-T changes occurred within the same saturation range. The deviation was, on the average, 2.4%. On administration of nitroglycerine the tolerance of the myocardium to hypoxemia increased, the S-T depression occurring only at considerably lower degrees of saturation and normalization occurring more rapidly I therefore seems that calibrated hypoxemia test with continuous O_2 registration is capable of giving standardized and reproducible registration of the degree of coronary insufficiency This test will also permit the registration of the effect of medication on *coronary insufficiency*

Inadequate coronary flow results in a number of metabolic and hemodynamic changes, which in turn result in an inadequate production of aerobic energy

Electromicroscopic studies of rats (7) have demonstrated pathological changes of the myocardium on even moderate hypoxemia. Changes in the mitrochondria could be demonstrated on respiration in 9% O atmosphere. Partial necrosis of the musculature occurred at 5% O_2 respiration.

Ischemia results in a modification of the action potential and of the K transport through the cell membrane (10).

Changes in potential can be revealed by ECG particularly by the T wave and the S-T interval The criteria of ischemic ECG changes ha e been discussed by many authors (4 8 14 15 20 22 23). The major criterion is the depression of S-T The reduction of the height of the T wave in hypoxemia was first demonstrated by Green and Gilbert in 1921 (9) In normal subjects breathing air poor in oxygen (down to 6%) they found a marked reduction in the T amplitude which sometimes became diphasic but not negative. Levy et al. (14 15) found depressed T amplitudes in normal subjects after 10% O respiration for 20 min. As this effect was often not reproducible they considered that it might be due to psychic causes Other workers ha confirmed that the T wa is reduced in normal subjects in hypoxemia (12, 16, 21). This T reduction has been observed in normal subjects even at *an arterial saturation of between 70 and 80%* (17)

A number of contradictory observations ha e been made. Larsen (13) was unable to find a reduction in the height of the T wa e in hypoxemia. In anoxia of the myocardium, which occurred in the early phase of open heart surgery (1) high, pointed T waves have been observed after only a few minutes. Wasserburger and Corliss (24) have collected a number of cases of infarction with initial appearance of an extremely high T wave in the precordial leads. They consider this to be due to a sudden shift of the intracellular potassium. In experiments with dogs, in which inhala-

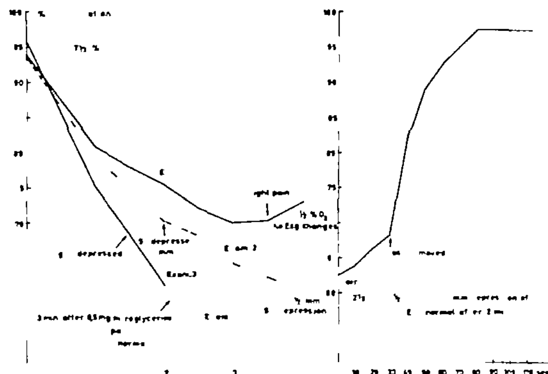


Fig 1 Hypoxemia tests in patient 26 with moderate angina pectoris.

tion of 10% O_2 was continued for several hours, Randall (18) often found a T elevation which was rapidly normalized on breathing ordinary air. Burman et al. (6) working with dogs, also found that the respiration of 9% O_2 resulted in a gradual elevation of the T waves, the maximum being reached after 4–8 min. In none of the animals was a reduction of the T wave found. The arterial O_2 saturation varied in some cases falling as low as 1 . The serum potassium rose from 4 to 5 7 mEq 1 and the pH fell to as low as 7 21 It seems reasonable to relate the ECG changes to the potassium shift.

In both healthy persons and coronary patients Barach et al (2) found a T elevation after respiration of pure O

The Q-T interval is also prolonged during hypoxemia, as Bellet and Finkelstein (3) demonstrated for the first time in 1951 Roehm et al. (19) have shown that the corrected Q-T interval is significantly more prolonged during the hypoxemia test in angina pectoris patients than in normal subjects. On the other hand, Kassebaum et al. (11) consider that the relative Q-P interval is of no importance in the diagnosis of coronary insufficiency

MATERIAL AND METHODS

The methods have been described in an other publication (5).

The material consisted of 5 normal subjects and 11 coronary patients, 6 with a light degree of angina pectoris, the others moderate. The arterial O saturation and the ECG in the precordial leads V–V was registered continuously before and during the test. The patients respired from an open mask, which was connected to a gas mixture of 7 % O in nitrogen One patient was given 8 / % O_2. The hypoxemia lasted from approximately 1 to 7–8 min.

RESULTS

Normal subjects

A reduction of the T elevation was found during hypoxemia in three subjects. Two showed no changes In one of these the arterial saturation did not fall below 80% the other was difficult to evaluate due to a technical disturbance in the ECG

The average reduction in the T wave was $^1/_2$– 3 mm. This reduction could be registered in all leads, but to a somewhat differing degree The changes came after 1–2 $^1/_2$ min, at an arterial saturation between 70–75% At the end of the test the mask was abruptly removed from the

Table I. *S-T changes and arterial oxygen saturation*

Case no	Test no	7½% O_2 respiration — Arterial O_2 saturation at beginning of S-T depression ( )	Free air respiration — Arterial O_2 saturation at normalization of S-T ( )
9	1	72	85
	2	72	85
	3	77	87
14	1	80 (after 3 min)	Not normalized at 94 saturation (after 33 sec)
21	1	69	S-T still depressed 4 min after
	2	71	full saturation. Normalized
	3	62 (after nitroglycerine)	3 min after full saturation
26	1	70	S-T normalized 2 min after
	2	63.5	full saturation
	3	52.5 ECG normal (after nitroglycerine)	
28	1	73	S-T normalized 30 sec after
	2	71	full saturation
29	1	No S-T depression at 47	
	2	Interrupted after 1½ min	
	3	Light depression (?) at 49 Interrupted after 1½ min	S-T normalized 20 sec after full saturation
30	1	61	S-T normalized at full
	2	56	saturation

subject, who then respired in ordinary air In all subjects the T wave became normalized 5–30 sec after the O_2 saturation began to rise. As a rule the saturation rose to normal values in the course of barely 30 sec. Normalization of the T wave took place in the range of 84–88% arterial saturation.

In none of the normal subjects examined was there any change in the S-T interval.

Coronary patients

T amplitude. In 11 patients there was a reduction of the T amplitude. In one patient (no. 20) there was no definite reduction of the T amplitude the O_2 saturation fell to 74%. The reduction showed the same pattern as in normal subjects and oc curred at different degrees of saturation, in one as early as at 90% saturation in two cases at 85%. The reduction was somewhat more marked at lower saturations.

The maximal reduction was 6 1/ mm. No inversion of the T wave was observed.

In cases in which the test was repeated the reduction of the T wave followed the same pattern. In two cases three tests were carried out, with the same results.

S-T changes. Marked changes in the S-T similar to those seen in coronary ischaemia oc curred in six patients. In one other subject (no 29) the test had to be int rrupted after only $1\,^1/_2$ min, as the oxygen saturation dropped to a ery low level. The ECG showed disturbances and the S-T interval was difficult to e aluate

Five of the patients in whom no S-T change was observed were light angina pectoris cases In one of them the O saturation did not fall below 80% whereas in three of the others the level was lower than 70%. In one test evaluation was difficult due to disturbance in the ECG

In six patients who showed S-T changes the minimum value of the arterial O_2 saturation ranged from 67 to 55%. The S-T depression oc curred at a saturation varying from 80% to 56%.

In two cases (nos. 17 and 29) the O_2 saturation fell very rapidly and the mask had to be removed after 1–2 min. In case 29 the saturation fell to 47%. The patient was restless and the ECG was difficult to evaluate. Both patients were clear cases of angina pectoris. It is obvious that a certain time must elapse before the changes appear the latent period would seem to be at least 1 $/_2$–2 min.

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FURTHER EXPERIENCES WITH KIDNEY PRESERVATION FOR 24 HOURS USING CONTINUOUS HYPOTHERMIC PERFUSION

Renal Clearances in Pigs with Autotransplanted Preserved Kidneys

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Abstract. Preservation of pig kidneys for 24 hours at 8°C has been studied using continuous perfusion with cryoprecipitated, microfiltered, oxygenated ACD-plasma. The perfusion pressure was about 65/30 mmHg and the flow from 1 to 2 ml/g kidney weight/min. Five consecutive experiments were performed. All the animals survived and were followed for three months. Measurements of inulin, endogenous creatinine, urea and PAH clearances 10, 31 and 94 days after transplantation showed normal values as compared to a control group of autotransplanted, not long-term preserved pig kidneys. The only permanent functional damage was a reduced maximal tubular excretion of PAH.

We have described previously our experiences with 24-hour preservation of pig kidneys using continuous hypothermic serum perfusion at a flow rate of 0.2 ml/g/min. Some of the preserved kidneys developed varying degrees of cortical necrosis postoperatively. These findings are in contrast to the results of other workers who, using hypothermic plasma perfusion at a greater flow rate than in our experiments, obtained consistently good results in dogs (1, 2, 10).

The purpose of the present work is to present results of hypothermic plasma perfusion of pig kidneys performed as previously described (7), but with the exception that the flow rate was increased to 1–2 *ml/g/min.*

MATERIAL AND METHODS

Five female pigs of the Danish Landrace breed, 4 to 5 months of age, and weighing 51 to 72 kg at the time of surgery were used for the study. The average kidney weight was 130 g (range 119–145). The animals were fed during the experimental period with a standard fodder mixture (3). They were weighed once a week, and the daily administration of fodder was calculated on the basis of the body weight. Unlimited quantities of water were permitted.

Preservation of the kidney

The kidneys were perfused in an apparatus constructed for clinical use by AB Gambro, (Lund, Sweden) in co-operation with Scandinavian transplantation centers (Copenhagen, Goteborg and Odense). The general principles have been described earlier (7), but due to the intended use in the clinic the system has been simplified and made completely portable (Fig. 1). The entire perfusion circuit is disposable (Fig. 2), and a new sterilized unit was used in each experiment.

The perfusion of the kidney was carried out by means of a roller pump producing a pulsatile flow. The pulse frequency was 20–40/min and the pressure curve is shown in Fig. 3.

Oxygenation of plasma was performed in a membrane oxygenator, in which the oxygen tension can be increased to about 600 mmHg. Due to separation of the circulation in the oxygenator and in the kidney (Fig. 2), it is possible to regulate the oxygen tension on the arterial side of the kidney by changing the flow rate in the oxygenator circulation. In our experiments the flow rate in the oxygenator circulation was about 100 ml/min, which gave an oxygen tension between 300 and 400 mmHg. The pH of the perfusate was controlled by varying the amount of CO_2 supplied to the oxygenator as described earlier (7), and was controlled 2 to 3 times during the perfusion. The temperature in the kidney was kept in the range of 7–9°C.

The urine produced during the perfusion was not separated from the perfusate, which was continuously filtered (Leuko-Pak® Leukocyte Filter) during the perfusion (Fig. 2).

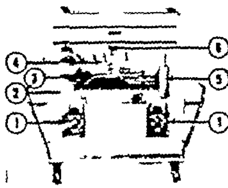


Fig 1 The perfusion apparatus, opened to visualize the main components. (*1*) Roller pumps in the organ and oxygenator circuits. (*2*) Membrane oxygenator (*3*) Cooling plate. (*4*) Organ container (*5*) Filter (*6*) Arterial pressure gauge

The *perfusate medium* was pooled homologous ACD-plasma ACD-blood (sol. citrate glucosi FbN ml 100 for stabilizing 400 ml blood) as centrifuged for 30 min t 000 rpm (1 326 g) The plasma was siphoned off and then stored at 20°C *Immediately before* use the *plasma* as thawed and *filtered* as described by *Belzer et al.* (*1*). Befo filtration 20 mg gentamycin NFN (Garamycin®) and 30 mg papaverine sulfas NFN were added to 700 ml plasma. Such as the volume used for each *perfusion*. Filtration of the plasma could b carried out within a few min, in contrast to our earlier xperiences with micro-filtration of serum, hen the filtration as often very slow procedure hich explains hy have changed f om serum to ACD plasma.

About half an hour befor removal of the kidney 500 ml 10 mannitol and 1 000 ml isotonic NaCl ere

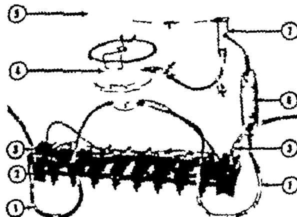


Fig 2. The disposable perfusion set-up (*1*) Organ and *oxygenator circuits.* (*2*) *Membrane oxygenator* (*3*) Oxygen inlet and outlet. (*4*) Organ container (*5*) Arterial pressure measuring outlet. (*6*) Filter (*7*) Bubble trap

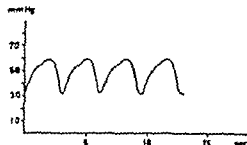


Fig 3 The pressure in the renal artery during the perfusion: flow 120 ml/min. Kidney weight 125 g.

given i. to the animals. Five min before nephrectomy the animals were *heparinized* (5 000 I.U heparin 10 kg b.wt.). As soon as possible after removal (2–4 min) the kidneys ere perfused with 200–300 ml TIS-U-SOL (6) and then placed in the apparatus. The *plasma perfusion* is *started and the flow rate regulated to give a perfusion* pressure of about 65/30 mmHg. The pH was adjusted as described above to 7.35 (measured at 37°C). T enty-four hours later the kidneys were removed from the perfusion circuit and reimplanted *in the animals*. The concentrations of sodium, potassium, standard bicarbonate and LDH in the perfusate medium was determined before and after the perfusion.

Surgical technique

Renal autotransplantation with uretero-ureteral anastomosis was performed. Nephrectomy as well as renal autotransplantation were performed by an extraperitoneal technique *and the preserved kidney wa placed at the site* of the opposite and just removed kidney using end-to-end anastomosis between the renal vessels Details are described lsewhere (9).

Peroperativ studies

Thirty minutes after recirculation renal blood flow as measured by means of the Xenon-133 ash-out technique (5). One hour after recirculation biopsy as taken from the kidney

Postoperativ studies

Blood analyses During the first eek after surgery blood *samples er taken every other day and subsequently* once eek for the following three months. The pH and haematocrit alues ere determined, together ith the concentrations in plasma of creatinine urea, sodium and potassium.

Kidney function. The clearances of inulin, endogenous creatinine urea, para-aminohippuric acid (PAH), and the excretion percentages of water sodium, potassium and chloride ere determined. These measurements were performed on unanaesthetized animals 10 31 and 94 days after transplantation. Each experiment comprised at least three periods of 20 min, and the last experiment concluded with three periods of 20 min to determine the maximal tubular excretion (Tm) for PAH. Three days after the last clearance experiment the percentage extraction of PAH was determined, after hich the animals ere killed.

Table I. *Biochemical data from five hypothermic perfusions*

Biochemical changes in the perfusate during the perfusion

Pig no.	Potassium (mEq/l) Start	Potassium (mEq/l) End	Sodium (mEq/l) Start	Sodium (mEq/l) End	Standard bicarb. (mEq/l) Start	Standard bicarb. (mEq/l) End	LDH (normal 44 µmol/h ml ± 6) Start	LDH (normal 44 µmol/h ml ± 6) End
129	11.3	11.0	145	145	15.3	23.0	28	—
137	4.6	7.1	157	150	22.0	30.1	38	59
138	5.1	7.9	154	149	15.1	20.3	35	44
139	10.4	11.7	143	143	13.6	19.6	47	46
141	4.1	6.0	152	149	13.8	13.6	38	59

Details on the doses of test substances, the technique and the calculation methods have been published previously (3).

Analytical methods have been described previously (4, 6, 7).

Postmortem examinations. After the observation period the animals were killed and bled, and postmortem examination was performed. The kidneys were weighed.

Histological examinations. At necropsy kidney tissue was removed and fixed in neutral buffered formalin. The biopsies taken one hour after recirculation were fixed in Zenker's fluid and in neutral buffered formalin. Paraffin wax sections were stained with iron haematoxylin-van Gieson, and the periodic acid Schiff reaction was carried out according to McManus and Mowry (8).

RESULTS

The perfusion

A perfusion pressure of about 65/30 mmHg during the perfusion was aimed at in all cases. Due to a decrease in the vascular resistance mainly during the first 3–4 hours of the perfusion, the flow rate was gradually increased. During the night the flow was kept constant and in all cases the pressure decreased somewhat (lowest value observed 35/15). During the last 3–4 hours of the perfusion the pressure was adjusted to the target values. At the start of the perfusion the flow rate in the different kidneys varied from about $\frac{1}{2}$ to 1 ml/g/min, but during most of the preservation period the perfusion flow rate was between 1 and 2 ml/g/min. The pH of the ACD-plasma used was about 7.0 before the start of the perfusion. After elimination of CO_2 in the oxygenator pH increased, and during most of the perfusion the oxygen supplied to the oxygenator was mixed with about 1% CO_2 to keep pH within normal limits. The standard bicarbonate concentration mostly increased during the perfusion (Table I). This is possibly due to metabolism of citric acid in the perfused kidney.

Initial behaviour

After removal from the perfusion circuit an average of 39 min passed before recirculation was established. Immediately after recirculation all the kidneys turned pink and became normal in consistency. This remained unchanged during the surgery. Urine production started within a few minutes and continued during the surgery.

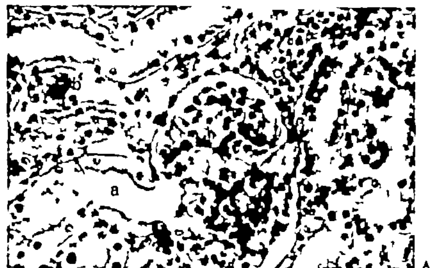
The blood flows of the preserved kidneys 30 min after recirculation are shown in Table II together with blood flow determinations in non-transplanted pig kidneys, measured by the same technique. It will be seen that no significant differences were found. B.P. was within normal limits in all cases.

Table II. *Renal blood flow in 24-hour preserved autotransplanted pig kidneys 30 min after recirculation compared with blood flow in non-transplanted pig kidneys*

Determined by means of Xenon-133 wash-out technique

24-hour preserved kidneys: Pig no.	Flow (ml/100 g/min)	Non-transplanted kidneys: Pig no.	Flow (ml/100 g/min)
129	70	92	266
137	152	93	73
138	175	94	112
139	94	95	246
141	158	96	120
		99	103
Mean 130 ± 43 (S.D.)		Mean 153 ± 81 (S.D.)	

0.50 $p < 0.60$



b
a
A



a
b
B



a
C

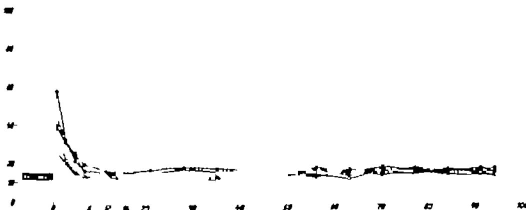


Fig. 5 Concentration of creatinine in plasma 0–94 days after transplantation. Ordinate: creatinine in plasma, µg/ml. Abscissa: days after transplantation. ○—○ pig 129 ●—● pig 137 — pig 138, ○- ○ pig 139 ● ● pig 141

The histological picture one hour after recirculation showed mostly normal conditions. However some characteristic features such as hyaline cylinders in the tubules (pigs 129 139 141 137) hyaline droplets in the cytoplasm of the tubular cells (pigs 129 139 141) neutrophils in the capillaries and interstitium (pigs 129 137 138 141) and slight dilation of the glomerular spaces (pigs 137 139) were found in varying degrees (Fig 4 A and B).

Subsequent function

All the animals survived the transplantation and were followed for three months.

Concentrations of creatinine urea, sodium and potassium in plasma. Figs. 5 and 6 show the concentrations of creatinine and urea in plasma (µg/ml) respecti ely The concentrations of sodium and potassium were constant throughout the period, 146 ± 5.8 (S.D) and 5.2 ± 0.7 (S.D) respec tively The pH remained constant, a eraging 7 42 ± 0.04 (S.D)

Fig. 4 (A) Histological section of kidney biopsy from pig 137 one hour after recirculation. Normal proximal tubule with distinct brush border (*a*). Distal tubule with hyaline cylinder (*b*). There are many neutrophils in the capillaries (←), in the interstitium and in the small veins (*c*). Some of the neutrophils contain PAS-positive granular material in the cytoplasm. Zenker's fluid. PAS reaction 320.

(B) Histological section of kidney biopsy from pig 139 one hour after recirculation. Dilated glomerular space (*a*). Hyaline cylinder in distal tubule (*b*). Hyaline droplets in the cytoplasm of proximal tubular cells (←). Neutral buffered formalin. PAS reaction. 320.

(C) Histological section of kidney from pig 138 three months after transplantation Normal tubules (*a*). Periglomerular fibrosis with histiocytes (←). Neutral buffered formalin. Iron haematoxylin – Gleason. 200.

Clearances of inulin endogenous creatinine urea and PAH Table III shows the a erage alues for clearances of inulin, endogenous crea tinine, urea and PAH, for the effective renal plasma flow ($RPF_{effective}$) and for the effective renal blood flow ($RBF_{effective}$). The clearance ra tios are stated.

Since the animals were killed immediately af ter the last clearance experiment, it was possible to calculate the clearance both per 10 kg body weight and per 100 g kidney tissue. The results are given in Table IV which also shows the extraction percentage for PAH used for calculat ing the total renal blood flow (RBF_{total}).

Maximal tubular excretion of PAH In the ex periments 94 days after transplantation the Tm of PAH was determined, and the results for each individual pig are shown in Table V which also shows the concentration of PAH in plasma at which the Tm was determined. The Tm values were calculated both per 10 kg body weight and per 100 g kidney tissue, and are on a erage 14 mg/min/10 kg body weight and 48 mg/min/100 g kidney tissue.

Excretion of water sodium, potassium and chloride The verage percentage excretion of water sodium, potassium and chloride are shown

Table III *Average renal clearances in five pigs 10–94 days after transplantation*

The figures in parentheses indicate minimal and maximal values of single experiments

D after transplantation	B.wt. (kg)	Hct (%)	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b.wt.)	Clearance: Endogenous creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)	Effective renal plasma flow (ml/min/10 kg b.wt.)	Effective renal blood flow (ml/min/10 kg b.wt.)
10	67	36 (31–40)	[illegible]5 (1.2–4.0)	21 (18–23)	21 (20–22)	10 (9–13)	76 (67–82)	83 (73–89)	1[illegible]9 (114–139)
31	86	40 (38–42)	1.8 (1.2–2.7)	19 (16–21)	21 (17–26)	11 (9–12)	68 (56–81)	74 (61–88)	1[illegible]3 (102–147)
94	127	43 (40–50)	2.0 (1.5–2.9)	20 (17–23)	[illegible]0 (17–22)	12 (10–17)	70 (56–84)	76 (61–91)	133 (107–160)

Table IV *Renal clearances and total renal blood flow in five pigs 94 days after transplantation*

Pig no	Hct (%)	Clearance: Inulin (ml/min/10 kg b.wt.)	Inulin (ml/min/100 g kidney)	Endogenous creatinine (ml/min/10 kg b.wt.)	Endogenous creatinine (ml/min/100 g kidney)	Urea (ml/min/10 kg b.wt.)	Urea (ml/min/100 g kidney)	PAH (ml/min/10 kg b.wt.)	PAH (ml/min/100 g kidney)
129	40	21	62	20	61	17	50	69	210
137	41	20	68	22	76	12	40	84	286
138	42	23	75	20	66	12	39	74	[illegible]44
139	42	19	74	17	68	10	40	56	[illegible]3
141	53	17	62	19	68	12	43	65	237

in Table VI. The average values for the whole period were 1.17 ± 0.58 (S.D.), 0.17 ± 0.15 (S.D.), 22 ± 8 (S.D.) and 0.73 ± 0.59 (S.D.), respectively.

Postmortem examination

Macroscopic findings. All the kidneys were normal in colour and consistency. Cortex and medulla had a normal appearance. Vascular and ureteral anastomosis were without complications.

Microscopic findings. Apart from minimal interstitial fibrosis in the kidneys from pigs 129, 138 and 139 no pathological changes were found (Fig. 4 C).

DISCUSSION

In the present study a perfusion technique closely similar to that originally described by Belzer et al. has been used (1). However, the use of cer

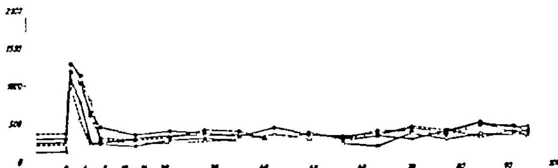


Fig. 6. Concentration of urea in plasma 0–94 days after transplantation. Ordinate: urea in plasma, μg/ml. Abscissa: days after transplantation. Symbols as in Fig. 5.

Clearance ratios		
Cr/In	Urea/In	Filtration fraction In/PAH
1.0	0.5	0.28
1.1	0.6	0 28
1 0	0 6	0.29

	Total renal blood flow	
Extraction (%)	(ml/min/10 kg b wt.)	(ml/min/100 g kidney)
86 0	146	442
88 0	176	599
88 0	158	520
92.2	114	453
85.5	158	576

tain additives (insulin, glucose, prednisone and magnesium) to the perfusate medium has been omitted.

Blood flow determinations by means of the Xenon-133 technique 30 min after recirculation showed great variations and revealed no significant differences as compared to a control group of non-transplanted kidneys (Table II)

The concentrations of creatinine and urea in plasma as well as different renal clearances were compared to the results of a control group of pigs with autotransplanted not long-term preserved kidneys (4)

The concentrations of creatinine and urea in plasma increased during the first day after transplantation (Figs. 5 and 6) fell after the second day and were stabilized within 7 days. This moderate increase in the concentrations of crea tinine and urea in plasma is similar to that seen in the control group. During the period from 7 t 94 days after transplantation the a erage con centration of creatinine in plasma was 14 g ml $\pm$ 1.3 (S D) which is equal to that of the control group.

The inulin, endogenous creatinine urea and PAH clearances on the 10th day after transplantation were higher in the long-term preserved group than in the control group ($0.01 < p < 0.02$) but on the 31st and 94th days no significant differences were found. *On the 94th day the* average values were 20 20, 12 and 70 ml min 10 kg b.wt., respectively while the values in the con*trol group were 18, 18 10 and 69 ml/min 10 kg* b.wt.

The excretion percentages of water sodium, potassium and chloride were nearly identical to the values obtained in the control group

The maximal tubular excretion of PAH was on average 14 mg/min/10 kg b.wt. $\pm$ 1.6 (S.D.) and in the control group 25 mg/min 10 kg b.wt. $\pm$ 4.4 (S.D.), ($p < 0.001$). While the preservation had no influence on the filtration and PAH clearances, nor on the blood flow of the kidney after three months, the estimation of the maximal tubular excretion of PAH shows that the pre servation has lowered this parameter The ex traction percentage of PAH was 89 $\pm$ 2 3 (S D), which is similar to the value in normal pigs (3)

Table V *Clearance and maximal tubular excretion (Tm) of para-aminohippuric acid 94 days after transplantation*

Pig no.	B.wt. (kg)	Kidney weight (g)	Relative kidney weight ( )	Plasma concentration of PAH (mg/ml)	PAH clearance (ml/min/10 kg b wt.)	Inulin clearance (ml/min/10 kg b wt)	Tm (mg/min 10 kg b wt.)	Tm (mg/min/100 g kidney)
129	140	462	0.33	1 410	24	14	14	44
137	125	348	0.29	1 310	28	16	15	50
138	137	414	0.30	1 240	30	17	16	53
139	134	337	0 25	1 620	21	14	12	48
141	137	375	0 27	1 540	22	14	13	44

Table VI. *Average renal excretion of water and electrolytes in five pigs 10–94 days after transplantation*

Days after transplantation	B.wt. (kg)	Diuresis (ml/min)	Inulin clearance (ml min/10 kg b.wt.)	Excretion (%) Water	Sodium	Potassium	Chloride
10	67	2.5	21	1.4	0.20	23.8	0.82
31	86	1.8	19	1.1	0.12	26.1	0.71
94	127	2.0	20	0.82	0.16	16.6	0.66

The average ratio between endogenous creatinine and inulin clearance was 1.04 ± 0.11 (S.D.) while it was 1.09 ± 0.14 (S.D.) in the control group ($0.15 < p < 0.20$).

The average filtration fraction was 0.28 ± 0.02 (S.D.) while it was 0.29 ± 0.05 (S.D.) in the control group ($0.20 < p < 0.25$).

In comparison to our earlier experiences with continuous low flow perfusions (7) the use of a higher flow rate significantly improved the results. Changes in the pre- and peroperative treatment of the animals (use of mannitol) and in the perfusate medium (changed from serum to ACD-plasma) are probably less important, since Claes et al. have found a similar difference between low and high flow perfusion when only this variable was changed (2).

When compared to our experiences concerning kidney preservation by hypothermia using a short initial cooling perfusion with a solution which mimics the intracellular ion composition (5) the continuous plasma perfusion caused a significantly smaller depression in renal function within the first 10 postoperative days. However, no differences in the renal function of the two groups could be demonstrated 30 days after the transplantation.

ACKNOWLEDGEMENTS

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FIBRINOLYTIC CAPACITY" IN HEALTHY VOLUNTEERS AT DIFFERENT AGES AS STUDIED BY STANDARDIZED VENOUS OCCLUSION OF ARMS AND LEGS

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Abstract. The local fibrinolytic response to standardized venous occlusion of arms and legs has been studied in 345 healthy volunteers of different ages (children, adults (18–50) and old people (above 65)). The fibrinolytic activity of resuspended euglobulin precipitate was measured on unheated fibrin plates. The fibrinolytic response to venous occlusion of the arms increased significantly (p 0.001) with age. In the aged the response was significantly ($p < 0.001$) lower in men than in women. In the legs the fibrinolytic response did not vary significantly with age below 50. The response was significantly lower in persons above 65 than in those below this age. The ratio between the response of the arm and that of the leg increased strongly with age. The results suggest that the predisposition of the legs to thrombosis and arteriosclerosis increases with age.

According to the view of Duguid (5, 6), who revived the early theories of Rokitansky (16), arteriosclerosis originates from undissolved mural deposits of fibrin, which become covered with endothelium and thereby incorporated in the vessel wall. Such deposits become organized and produce thickening of the intima, which is later infiltrated by lipids. The theory of Duguid has been adopted and extended by Astrup (1). He postulates that minute intravascular coagulation is a normal process. According to Astrup, small fibrin strands are being continuously deposited on the vascular endothelium and removed by fibrinolysis. Any depression of fibrinolysis would therefore upset this equilibrium, with continuous deposition of fibrin in the vascular lumen and consequent predisposition to thrombosis and arteriosclerosis.

Several objections have been raised against Duguid's and Astrup's theory (8, 19). It is, for example, known that arteriosclerosis does not spare haemophilic patients (8) or patients with von Willebrand's disease (18) in spite of their impaired coagulation. Furthermore, long-term treatment of animals with fibrinolytic inhibitors has not induced widespread deposition of fibrin in the vessels (13).

A clue to the solution of the problem may perhaps be obtained by studying the fibrinolytic system in subjects of different ages, since thrombosis and arteriosclerosis have become more common with increasing age. Several workers have studied the fibrinolytic system in patients with various manifestations of arteriosclerotic disease and in patients of different ages (2, 3, 7, 8, 11, 17, 20, and others). These investigations have yielded conflicting results. Some workers have found the fibrinolytic activity in aged people and in arteriosclerotic subjects to be decreased (2), while others have found it to be unchanged or even increased (3, 7, 8, 9, 17, 20).

A limitation of most of these studies is that they measure only the spontaneous fibrinolytic activity of blood, which is generally low and unsuitable for demonstrating individual variations of fibrinolytic activity. We found that the fibrinolytic response to venous occlusion of the limbs is a sensitive and reliable method for assaying the individual capacity to mobilize the endogenous fibrinolytic agents (fibrinolytic capacity) (14, 15). We thought it interesting to apply this method to healthy volunteers of different ages to see whether the fibrinolytic capacity varies with age.

MATERIAL

The clinical material consisted of 20 children (13 male and 7 female) aged 6–12, 177 men aged 18–24, 35 men aged 25–50, 49 women aged 18–30, 64 old persons aged 65–95 (23 male and 41 female) and living in three homes for the aged in the city of Malmö. All these 345 volunteers were apparently healthy and none of them had any

Table VI. *Average renal excretion of water and electrolytes in five pigs 10–94 days after transplantation*

D after transplantation	B.wt. (kg)	Diuresis (ml min)	Inulin clearance (ml min/10 kg b. t.)	Excretion ( )			
				Water	Sodium	Potassium	Chloride
10	67	2.5	21	1.6	0.20	23 8	0.8?
31	86	1 8	19	1 1	0 1	26.1	0.71
94	177	2.0	20	0.82	0 16	16 6	0 66

The average ratio between endogenous creatinine and inulin clearance was 1.04 ± 0 11 (S.D) while it was 1.09 ± 0 14 (S.D) in the control group ($0.15 < p < 0.20$)

The average filtration fraction was 0.28 ± 0.02 (S.D) while it was 0.29 ± 0.05 (S.D) in the control group ($0.20 < p < 0.25$)

In comparison to our earlier experiences with continuous low flow perfusions (7) the use of a higher flow rate significantly improved the results. Changes in the pre and peroperative treatment of the animals (use of mannitol) and in the perfusate medium (changed from serum to ACD-plasma) are probably less important, since Claes et al. have found a similar difference between low and high flow perfusion when only this variable was changed (2).

When compared to our experiences concerning kidney preservation by hypothermia using a short initial cooling perfusion with a solution which mimics the intracellular ion composition (5) the continuous plasma perfusion caused a significantly smaller depression in renal function within the first 10 postoperative days. However no differences in the renal function of the two groups could be demonstrated 30 days after the transplantation

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subjects under 50 years was 74±57 mm². In persons above 65 years of age the local fibrinolytic response to venous occlusion of the legs was significantly lower than in the younger groups ($p < 0.001$).

3. Variation of the ratio between the fibrinolytic response of the upper limb and that of the lower limb with age

Tables I and III show that in children the mean local fibrinolytic activity after venous occlusion was slightly more than twice as high in the arms as in the legs, in adults 3 to 4 times, and in old persons (> 65) 8 to 10 times.

DISCUSSION

As judged from the fibrinolytic response of arms to venous occlusion, fibrinolytic activity increased with age (Table II). These results do not confirm the findings of Buckell & Elliott (2) but they are in agreement with those of later workers in this field (3 7 8, 9 17 20), who found the spontaneous fibrinolytic activity either to remain unchanged or to increase with age.

In our material there was a difference in the fibrinolytic response to venous occlusion of the arms with sex (Table I). In the males the response did not increase with age after childhood, and the increasing fibrinolytic response in old age (Table II) was due to the strong fibrinolytic response in the old women. The response to venous occlusion of the legs was substantially unchanged up to the age of 50. For some unknown reason the response decreased after the age of 65 years. In this connection it might be mentioned that the quotient between the fibrinolytic activity after venous occlusion of the arms and the legs increases markedly with age. In children the activity was only twice as high in the arms as in the legs; in adults it was about 4 times as high, and in old persons (> 65) 8 to 10 times.

As is known, venous occlusion enhances the fibrinolytic activity in the blood by causing liberation of plasminogen activator from the walls of blood vessels. It might be assumed that in the older age group an existing subclinical venous stasis of the lower limbs due to several factors such as impaired venous muscle pump decreased heart function, sclerotic changes of the vessels, resulted in a chronic stimulation of the vessels with more or less decreased release of their fibrinolytic activator. This would result in a decreased fibrinolytic response of the vessels in the legs to experimental venous occlusion.

In previous papers (12, 14) it was suggested that the stronger local fibrinolytic response to venous occlusion of the arms than to venous occlusion of the legs is one of the reasons why thrombosis and arteriosclerosis are more common in the legs than in the arms. Our results show that this difference becomes more evident with age and thus increases the predisposition of the legs to thrombosis and arteriosclerosis. In this respect our results may lend further support to Astrup's (1) and Duguid's (5 6) thrombogenic theory of arteriosclerosis.

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LACTIC ACIDOSIS IN PHENFORMIN TREATED DIABETICS

A Clinical and Laboratory Study

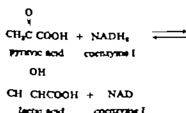
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Abstract. From a fairly large clinical material of diabetics treated with the antidiabetic drug phenformin 21 patients showed clinical and laboratory evidence of lactic acidosis. The diagnosis was confirmed by direct and specific determinations of lactate (L) and pyruvate (P) in 15 of the patients. All of them showed raised L and P levels with raised L/P ratio. All remaining six patients had severe metabolic acidosis with low pH values and increased so called "anion gap". Predisposing factors in patients developing lactic acidosis on phenformin treatment seem to be impaired renal and liver function, in some cases also alcohol consumption and interaction with other drugs. The mortality rate is high despite intensive treatment, in the present material 33%. It is concluded that phenformin therapy is contraindicated in diabetics with impaired renal or liver function. The treatment dose also seems to be of importance. The prognosis depends on early diagnosis and intensive treatment with bicarbonate intravenously probably also in combination with administration of insulin and glucose. The exact role of phenformin in causing lactic acidosis is not known, but probable mechanisms are discussed.

Lactic acidosis as a biochemical and clinical entity was first described by Huckabee in 1961 (7). In recent years this entity has been more commonly observed and has been reported to occur also in connection with phenformin treatment of diabetes (4, 5, 6, 9, 11, 14, 20). Up to 1968 at least 111 cases had been published according to Braaten and Hansen (2), although only 58 were diabetics.

Lactic acidosis may develop because of anaerobic metabolism according to the following formula.



Over the physiological pH range lactic acid exists predominantly as ionized lactate and pyruvic acid as pyruvate.

In blood the normal ratio between lactate (L) and pyruvate (P) is 10:1 (upper limit). In many physiological conditions (i.e. physical exercise) there is a raised blood lactate level with a proportional rise of the pyruvate level. In some conditions, however, the rise of L is unproportionately high and the L/P ratio exceeds 10:1.

Hyperlactatemia may be primary or secondary (7, 11, 13). Secondary hyperlactatemia may occur in serious clinical conditions connected with tissue hypoxemia, i.e. profuse hemorrhage, septicemia and myocardial infarction. Hyperlactatemia without known predisposing factors is called primary or spontaneous (7).

The aim of this paper is to report our experiences from a fairly large clinical material. Part of the material has been published earlier (11).

MATERIAL AND METHODS

The clinical material comprised 21 patients seen at the Regional Hospital, Linköping, Sweden, between Oct. 1967 and Jan. 1971. Age and sex of the patients are shown in Fig. 1. The typical clinical picture was outlined as follows:

Onset: Fairly rapid, one to a few days.

Symptoms: Poor appetite, low food intake, nausea, vomiting.

Signs: Increasing hyperventilation, no smell of acetone. Cerebral confusion, coma. Hypovolemia, lowered blood pressure, oliguria, anuria. Death due to circulatory collapse.

Type of diabetes and duration of the disease are shown in Table I. Antidiabetic therapy, maintenance dose of phenformin and duration of therapy are seen in Table II.

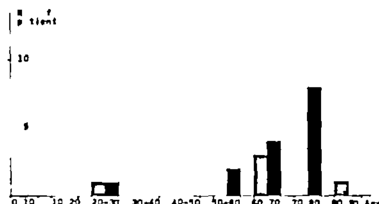


Fig. 1. Age and sex of 21 patients with lactic acidosis. ☐, men; ■, women.

Determinations of pH, pCO and bicarbonate were made in capillary blood by routine laboratory methods and the base excess (BE) was calculated. Capillary blood sugar was measured by the glucose oxidase method. Venous blood L and P were determined enzymatically according to Scholz et al. (14). Normal values in our laboratory are: Lactate 6–16 mg 100 ml = 0.67 – 1.78 mM/L. Pyruvate 0.4 1... mg/100 ml = 0.05–0.14 mM/L.

If methods for determination of L and P are not available, lactic acidosis may be suspected by the presence of so called anion gap". This can be calculated as follow (N)–(Cl + HCO) = anion gap (24). The difference should normally not exceed 1 mEq/L.

RESULTS

Clinical

Of the 21 patients 7 showed clinical signs of impending shock. Of these, however only two systolic blood pressures below 90 mmHg.

In 9 patients there were signs of dehydration h dry mucous membranes and decreased skin turgor. Twelve patients showed signs of hyperventilation without any recognizable smell of acetone.

Mortality

Seven of the 1 patients died, a mortality rate of 33%. Of these however two showed signs of acute myocardial infarction at autopsy whereas in the other five death was probably due entirely to the lactic acidosis per se. In these five patients autopsy failed to reveal any other cause of death.

Laboratory findings

The initial values for pH are shown in Fig. 2. In every case the value was below 7.3 and in eight patients extreme values of pH below 7.0 were found. The lowest recorded pH value was 6.84 (2 pats.)

BE values for the patients are shown in Fig. 3. All patients had BE values below –10, and ten patients had values below –22, which is the lowest measurable value for the method used.

In 15 and 13 patients, respectively direct measurement of *L and P serum levels* were performed according to the methods previously men-

Table I. *Type of diabetes mellitus and duration of disease (y.)*

Age at onset	N of pats.	Range	Mean
Infantile (0–14 y.)	1	19	
Juvenile (15–.4 y.)	1		
Adult (25–64 y.)	13	27	9
Old ge (> 65 y.)	6	‡–6	3

Table II. *Antidiabetic therapy maintenance dose and duration of phenformin therapy*

	No. of pats.
Antidiabetic therapy	
Phenformin	4
Chlorpropamide phenformin	9
Insulin phenformin	6
Tolbutamide phenformin	1
Tolazamide phenformin	1
Maintenance dose of phenformin	
50 mg/d.	5
100 mg d.	15
U known	1
Duration of phenformin therapy	
½ y	5*
½–2 y	6
2 5 y	5
Unknow	5

One pat. 3 days.

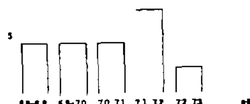


Fig 2 Lowest recorded pH values in 21 patients with lactic acidosis.

tioned. As shown in Fig. 4 all measurements revealed elevated L, sometimes to extreme values (above 100 mg/100 ml) and a much elevated L/P quotient in all cases.

The renal function, as judged by serum creatinine levels, is illustrated in Fig. 5 The values recorded are the highest and lowest for each patient during the hospital stay As a rule the highest level was recorded on admission. As is shown in the figure, most of the patients had impairment of renal function initially and ten of them had persisting high creatinine levels, indicating permanent renal damage.

Liver function tests were performed in 14 patients in the acute situation. Nine had normal whereas five had moderately elevated serum enzyme (S-GOT S-GPT and S-LDH) values. In two of these patients, however autopsy showed myocardial infarction.

Blood sugar levels for the material are shown in Fig. 6. Hypoglycemia was found in seven of the patients, with as low values as 22 mg/100 ml in one and 32 mg/100 ml in two.

The "anion gap" is shown in Fig. 7 Marked elevations were found in every instance

Treatment

Therapy has been intravenous administration of 0 6 M bicarbonate. The total amount of infused bicarbonate varied between 120–1 140 mEq, mean 562 mEq (Fig. 8)

DISCUSSION

The exact mode of action of phenformin is unknown. Steiner and Williams (18) found that phenformin had a suppressive effect on certain enzymes in the citric acid cycle, leading to increased anaerobic glycolysis. They also showed that phenformin in vitro increased the glucose uptake in the cell and decreased the glycogen storages (19). Suppression of the gluconeogenesis in the liver has also been discussed (17)

The so called spontaneous lactic acidosis (Huckabee type 2 B) occurs at a higher frequency in diabetics than in non-diabetics.

Although several factors indicate that phenformin may cause lactic acidosis, it is not fully proved that this is the case. In 1960 Craig et al. (3) showed that phenformin-treated diabetics had increased resting values of blood lactate. This was subsequently verified by Bernier et al. (1) Although these authors could seldom demonstrate values above 3 mM/l at rest, phenformin treatment for one week in a dosage of 150 mg daily did not interfere with the ability to metabolize excess lactate produced by muscle work.

According to Craig et al. (3) the lactate level in phenformin-treated diabetics should depend on the serum level of phenformin.

The duration of phenformin therapy does not seem to be of any importance in the development of lactic acidosis, as the time interval between the beginning of phenformin treatment and the appearance of lactic acidosis varies between three

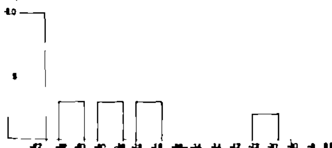


Fig 3 Lowest recorded BE values.

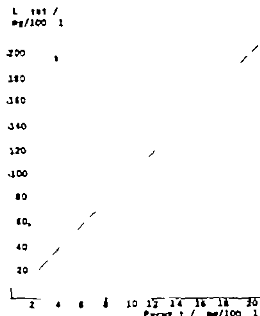


Fig 4 Lactate and pyruv te alues, patients, — — the normal L P ratio, 10 1

days up to six years in our material. The same opinion has been offered by Oliva (12) It has also been suggested that phenformin per se could not lead to development of lactic acidosis but rather that its own metabolic effects may aggravate lactic acidosis caused by other factors. Lieber and Davidson (10) showed that ingestion of ethanol increased the blood lactate Johnson and Waterhouse (9) could verify that this lactate increase was potentiated by phenformin and they concluded that excessive consumption of alcohol should be avoided in phenformin-treated patients. Lactic acidosis as a result of interaction between phenformin and other drugs, for instance allopurinol, has also been discussed (?0)

The *renal* function deserves the utmost attention before introduction of phenformin treatment, because the substance and its metabolites are excreted by the kidneys (13). In some cases it has been reported (22) that even a moderate increase of the creatinine levels to 2–3 mg/100 ml has been associated with lactic acidosis. In these patients phenformin concentration in plasma has

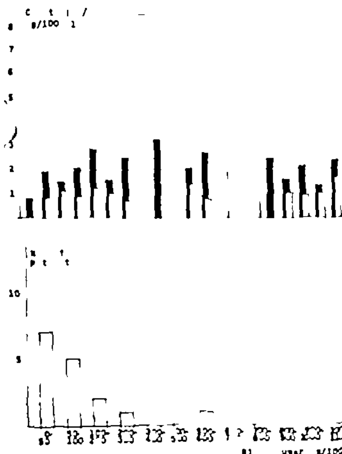


Fig 5 Renal function as measured by serum creatinine The figure shows the highest and lowest recorded alues for each patient. Four patients died before the second value could be obtained.

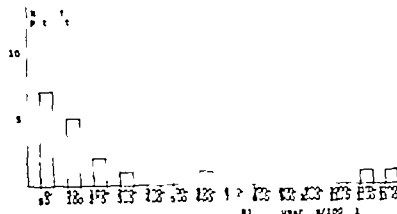


Fig 6 Blood sugar alues in the acidotic phase



Fig. 7. Anion gap = Na⁺ − (Cl^- + HCO_3^-).

been increased three to four times above the level observed in patients with normal kidney function. Thus it seems that accumulation of phenformin may occur in patients with impaired renal function. Before treatment is started, determination of the creatinine clearance should be performed. Impaired renal function must be regarded as a relative contraindication to treatment with phenformin.

In our material the serum creatinine level on admission was increased (1.35–8.3 mg/100 ml) in 20 patients. In connection with adequate treatment of the lactic acidosis the creatinine level has returned to normal in some patients, whereas in others persisting elevated creatinine levels have been demonstrated, indicating a preexisting renal damage. The initial rise of the creatinine level in some patients seems to have been due to dehydration, secondary to anorexia and vomiting.

Onset and clinical picture

Lactic acidosis is said to begin abruptly within hours. In our experience, however, most patients have had prodromal symptoms for a couple of days. These symptoms are mainly drowsiness, anorexia and nausea. Secondarily there is inadequate intake of food and fluids, leading to dehydration and contributing to low blood sugar values. On admission the patients as a rule exhibit severe acidosis and in many cases shock or preshock symptoms. In our material this occurred in seven cases. Accompanying the lactic acidosis a ketoacidosis may be found and it has been proposed that diabetics with ketosis are more prone to develop lactic acidosis than patients without ketosis (13). With coexisting ketosis Shreeve et al. (16) demonstrated lowered tolerance to exogenous lactate administration.

The laboratory pattern in lactic acidosis is dominated by the severe metabolic acidosis. The elevation of the serum lactate is proportionally higher than the elevation of the pyruvate level. The anion gap is pronounced. Ketonemia and ketonuria are sometimes seen but are often lacking. If methods for determination of lactate and pyruvate are not available, the diagnosis can be suspected by an increased anion gap in the absence of other causes of acidosis, e.g. ketosis, renal insufficiency, salicylate or methanol intoxication.

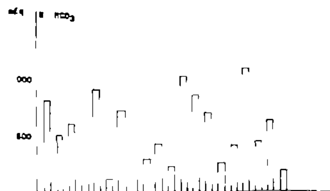


Fig. 8. Total amount of sodium bicarbonate infused in each patient to correct acidosis.

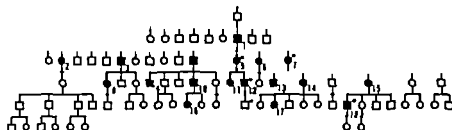


Fig 1 Pedigree of family A. □, male ○ female ■ affected, complement factors analysed. Numerals to the right of the symbols refer to case number

be ascribed to activation of a serum globulin permeability factor and/or kallikrein in serum. The $C\bar{1}$ INH was found to inhibit, besides $C\bar{1}$ also plasma kallikrein and plasma permeability factor (Pf/dil) (18 24)

Plasmin also splits kinin from serum proteins (7). Ratnoff et al. (46) claimed that the $C\bar{1}$ INH inhibits also other serum enzymes, i.e. plasmin. The possibility may be considered that, besides kallikrein, plasmin also may play a role in the precipitation of the symptoms in HANE. However in one patient studied by Lundh et al. (37) no activation of plasmin was found. No oedema is seen when the formation of plasmin is strongly increased, for instance by injection of streptokinase

Increasing interest has been concentrated upon the possibility of kinins eliciting the symptoms in HANE. Recently Donaldson et al. (15) have partially characterized a small molecule from HANE plasma which caused a markedly increased vascular permeability Apparently it is a polypeptide but differs from known kinins in several ways New approaches concerning the treatment of HANE have been published during recent years. In the present paper the findings on complement analysis of serum and plasma from members of three families with HANE are reported and variations of the symptomatology and results of treatments are discussed.

MATERIAL AND METHODS

Blood was sampled from the patients and serum centrifuged off within 2 h. The sera were stored in small aliquots at −80°C until analysed.

C1-esterase ($C\bar{1}$). Euglobulin of normal human serum was prepared as described by Lepow et al. (34). $C\bar{1}$ was activated by incubation of the euglobulin at 37°C for 15 min.

N-acetyl-L-tyrosine-ethyl ester (ATEe) was obtained from British Drug Houses, Poole, England.

C1-esterase inhibitor ($C\bar{1}$ INH) of serum was determined by (*a*) Estimation of the inhibition of the ATE hydrolyzing capacity of $C\bar{1}$ according to Levy and Lepow (35) by pH stat titration as described by Laurell and Sabon (31). Normal range 19–29 U/ml. Mean value 45 U/ml (29). (*b*) Electrophoresis in agarose gel containing antibodies as described by Laurell et al. (29). Normal range 70–113% of normal standard pool.

C1-esterase ($C\bar{1}$) of serum was determined described by Laurell et al. (30).

Total C was determined according to Pillemer et al (44). Normal range 90–154 U/ml.

C4 was determined by (*a*) The immune haemolytic technique according to Pillemer et al. (44). Normal range

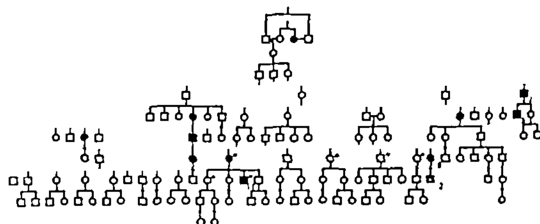


Fig. Pedigree of family B Symbols as in Fig. 1

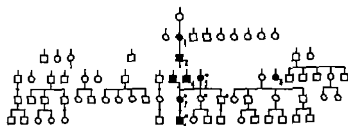


Fig. 3. Pedigree of family C. Symbols as in Fig. 1.

600–1 200 U/ml. (b) Electrophoresis in agarose gel containing specific antiserum (32). Normal range 40–200% of normal standard pool.

C3 was determined by electrophoresis in agarose gel containing specific antibodies (36). Normal range 60–140% of normal standard pool.

Antigen-antibody crossed electrophoresis according to Laurell (33). A specific antiserum (29) was used for analysis of the $C\bar{1}$ INH.

CASE REPORTS

Three families with together 21 now living and 17 deceased members with HANE are described. The

Table I. *Three families (A, B and C) with HANE*

Case no.	Sex	Born	Deceased	Cause of death	Age when symptoms started (y)	Complement factors analysed
A 1	♂	1850	1908	Asphyxia	9	
2	♀	1880	1902	Asphyxia	5	
3	♂	1886	1943	Asphyxia	20–25	
4	♂	1893	1967	Heart disease	16	
5	♀	1897			7	+
6	♀	1900			8	
7	♀	1903			20	+
8	♀	1921			1	
9	♂	1922			12	
10	♂	1931			2	
11	♀	1920			6	
12	♂	1922			6	+
13	♂	1924			15	
14	♀	1931			19	
15	♀	1932			15	
16	♀	1961			4	
17	♀	1952			1	
18	♂	1949			½	+
B 1	♀	1736	1781	"Swelling		
2		1810	1863	Asphyxia		
3	♀	1834	1896	?	< 5	
4	♀	1835	1895	Disease of albumin		
5		1841	1868	Dropsy		
6	♀	1866	1901	"Disease of albumin		
7		1870	1931	Asphyxia	< 5	
8	♀	1892	1922	Pneumonia	< 5	
9	♀	1897			6	−
10	♀	1914			6	+
11	♂	1928			7	+
12	♂	1937			7	+
C 1	♀	1840	1879	Peptic ulcer disease		
2	♂	1872	1926	"Gallstone	16	
3		1895	1912	Asphyxia	< 5	
4	♂	1897	1924	Asphyxia	< 5	
5	♀	1899			9	+
6	♀	1903	1963	Cancer mam	16	
7	♀	1922			4	+
8	♂	1931				

Table II. Symptoms during attacks of HANE in living members of families A, B and C

Case no.	Prodromal symptoms	Peripheral oedema	Laryngeal oedema	Abdominal pain	Diarrhoea	Exanthema	Joint pain	Fever	Abnormal thirst
A 5				+	+				
6			−		−				
7	−		−	+	−				
8					−	(+)			
9					+		(+)		
10				+	−				
11					+				
12					+	(+)			
13	−	+		( )	+				
14		( )	−		+		( )		( )
15					+				
16	−	( )	+			+			
17	−		( )	−					
18				( )	−				
B 9									
10		+							
11	−	( )		+					
12									
C 5									
7									
8					−				

pedigrees of these families are shown in Figs. 1 3. With the aid of genealogic experts these families could be traced back as far as nine to eleven generations. Families A and C have lived in the County of Bohuslän and family B in the County of Västergötland in Sweden at least since the 17th century. No connection between the three families could be traced.

Data and symptoms of the affected members of the lies are given in Tables I-III. For deceased persons relatives have related the symptoms. The influences f pregnancy and menstrual period on the symptoms are shown in Table IV. Some of the older HANE patients re now free from symptoms. A discussion of some patients of peculiar interest ll be given below.

Family A

Case 5 had severe attack of HANE every fortnight from early childhood to 65 years of age. The attack ceased about the same time as symptoms of diabetes mellitus appeared. During four y ars she was treated only with carbohydrate-reduced food. A the age of 70 insulin therapy was started. One year later the HANE symptoms started again, appearing every fortnight. The ttack were however not as severe as arlier.

Case 7 has spontaneously been free from HANE symptoms since 1965. Diabetes mellitus was diagnosed in 1969.

Case 8 and case 10. These tw HANE patients were reported by Arnoldsson et al. (4). In 1964 diabetes mellitus was diagnosed in case 10. H has since then been free from his earlier severe ttack of HANE. Case 8 has severe symptoms with only short intervals.

Case 12 was e rlier described by Arnoldsson et al. (4) and Granerus et al. (19). The dominating symptom s severe abdominal pain with nausea and vomiting every fortnight. During the attacks there was severe heartburn and he was hospitalized several times for peptic ulcer disease with haemorrhage. Since childhood he had suffered from attacks of abdominal distress every fortnight. He had to stay at home from school and later from his work for 1 days on such occasions. EACA (epsilon-aminocaproic acid) therapy was started in the spring of 1967. During the first two months EACA was given intermittently in a dose of 6-30 g daily at the first sign of symptoms. After two months of intermittent EACA therapy he was treated continuously with dose of 3 g EACA twice daily. The dose was raised to 6 g EACA 4-5 times daily every fortnight when prodromal symptoms ppeared. During one year of this regimen only one or tw attack of abdominal pain occurred. Since the summer of 1968 EACA was again administered intermittently in dose of 6 g 4-5 times daily for 1 2 d ys every fortnight at the first sign of prodromal symptoms. Since then he has only had two attack of abdominal d stress. On neither occasion had EACA been given prior to the attack. When the attack had started the drug could n be retained owing to vomiting. The abdominal sympt ms were most dramatically affected by EACA ther py. vers the peripheral oedema appeared less frequently nd s less widespread. The severe heartburn disappe red the treatment and has not reappe red in the last ye rs. H has not noticed any side-effect of EACA.

Case 13 had attack of oedema since the ge of 3 months. The frequency of ttack has been 4 4 monthly during the last ye rs, they h s ppe red at regular intervals. Occasionally an ttack could be pre

Large volume of urine	Peptic ulcer disease	Icterus	Head-ache	Mental symptoms
	(+)			+
	(+)		+	+
	(+)		+	
+	(+)			+
	+			
				+
+	+		+	+
+				+
+	(+)			
				+
	(+)			
	+			+
	+	+	+	+
	(+)			+
	+	+		+
	(+)		+	
	+		+	+

by blow About three times year he suffered from oedema of the larynx. Gastrointestinal attacks ere combined with loss of appetite, abdominal swelling and tenderness but not nausea or vomiting. Flatulence but not diarrhoea appeared after the attacks. Heartburn was often present during but not between the attacks. The basal and histamine stimulated secretion of hydrochloric acid was normal when tested between two attacks. EACA in dose of 6 g five times daily was tried, but the therapy was interrupted because of nausea before any statement of the effect on the HANE symptoms as possib e.

Family B

Case 5 had attacks of abdominal pains and omiting since childhood. No signs of peripheral oedema hav been reported. She was icteric during some of her attacks and hen she died.

Case 10 had attacks of HANE 1–4 times per month since the age of six. Trauma might initiate an attack, but never during the first week after an attack. The attacks consisted of peripheral oedema, abdominal pains with nausea, vomiting and diarrhoea, paralyzing tiredness and mental depression. Sometimes the body temperature was increased and occasionally there ere pains in the joints. She had observed jaundice and dark urine during few attacks. X-ray in 1957 showed normal gallbladder

She was admitted to hospital in 1965 with severe haemolytic crisis which appeared 4–5 day after the start of an attack of HANE ith abdominal pains, vomiting,

Table III. *The variability of attacks of HANE in living members of families A B and C*

Case no.	Peripheral oedema and abdominal pain occurring in the same attack: Frequently	Sometimes	Rarely	The most frequent symptom: Peripheral oedema		Abdominal pain	The length of an attack (days)	Interval between attacks at the most severe stage of HANE (weeks)	Periodicity of attacks
A 5		+				+	2–4 (–8)	1–2	
6	+			+			3 (–7)	2	
7						+	?	2	
8	+			+	–	+	3–7 (–30)	0	
9	+			+			2 3 (–5)	2 5	–
10		+		+	–	+	2–4	1	
11		+		+			2–4 (–8)	1 2	
12	+					+	1 2 (–3)	1 2	
13			+	+			2–4 (–7)	1–2	
14			+			+	1 2 (–3)	1 3	( )
15		+		+			2 5 (6)	2–4	
16			+			+	1–3	4–8	
17				+			?	4–8	
18		+		+			1–3 (–5)	2–4	
B 9	+					+	1 (–5)	1 2	
10						+	1 (–3)	1	
11			+			+	1 (–2)	1	–
12						+	4–10 (14)	0	
C 5				+			1 (–2)	2	
7	+			+			1–3 (4)	1	
8									

Table II. *Symptoms during attacks of HANE in living members of families A B and C*

Case no.	Pro-dromal symptoms	Peripheral oedema	Laryngeal oedema	Abdominal pain	Diarrhoea	Exanthema	Joint pain	Fever	Abnormal thirst
A 5	+		+	+	+				
6	+		–	+	–				
7	–		–	+	–				
8				+	–	(+)			
9			+		+		(+)		
10	+			+	–				
11				+	+				
1	+	+		–	+	(+)			
13	–	+	+	( )	+				
14	–	( )	–		+		(+)		( )
15		–	+	+	+				
16	–	( )	+	+	–	+			
17	–	+	( )	–	+				
18				( )	–				
B 9			+	+	+				
10	+	+	+	–				+	
11	–	( )		+					
1		+		+	–			+	
C 5				–					
7					–				
8				–	–				

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Case 7 has spontaneously been free from HANE symptoms since 196 Diabetes mellitus as diagnosed in 1969

Case 8 and case 10 These two HANE patients ere reported by Arnoldsson et al 4) In 1964 diabetes mellitus was diagnosed in case 10 He has since then been free from his earlier severe attack of HANE. Case 8 has severe ymptoms with only short intervals.

Case 1 was earlier described by Arnoldsson et al (4) and Granerus et al. (19). The dominating symptom as severe abdominal pain with nausea and vomiting every fortnight. During the attacks there was severe heartburn and he was hospitalized several times for peptic ulcer disease with haemorrhage. Since childhood he had suffered from attacks of abdominal distress every fortnight. He had to stay at home from school and later from his ork for 1 days on such occasions. EACA (epsilon-aminocaproic acid) therapy was started in the spring of 1967 During the first tw months EACA was given intermittently in a dose of 6–30 g daily at the first of symptoms. After two months of intermittent EACA therapy he was treated continuously with dose of 3 g *EACA twice daily The dose was raised to 6 g EACA* 4–5 times daily every fortnight when prodromal symptoms ppeared. During one year of this regimen only one of two ttacks of abdominal pain occurred. Since the summer of 1968 EACA was again administered intermittently in a dose of 6 g 4–5 times daily for 1 days every fortnight at the first sign of prodromal symptoms. Since then he has only had two attacks of abdominal distress. On neither occasion had EACA been given prior to the attack. When the attack had started the drug could not be retained owing to vomiting. The abdominal symptoms were most dramatically affected by EACA the gh even the peripheral oedema appeared less frequently and s less widespread. The severe heartburn disappeared under th treatment and has not reappeared in the last / years. H has not noticed any side-effects f EACA.

Case 18 had attack of oedema since the age of 3 months. The frequency f attack h been 4–6 weekly monthly during the last years, they ha not appeared at regular intervals. Occasionally an ttack could be provoked

of them (A 5) symptoms of HANE reappeared six years later It may be that the metabolic disorder in some way interferes with the symptoms of HANE.

In family B there is an accumulation of jaundice, pernicious anaemia and hypothyreosis. Case B 3 during many years had attacks of jaundice which were related to abdominal distress. Case B 10 had jaundice during some HANE attacks, also before she had the attack of severe haemolytical jaundice described. The haematological investigation indicated a slight vitamin B_{12} deficiency On vitamin B_{12} therapy there has been no further attack of jaundice, though the HANE symptoms were not influenced. Her son (B 12) has been hospitalized several times for a juvenile "non-haemolytic jaundice. H has normal serum B_{12} values and no correlation between periods of HANE symptoms and periods of jaundice.

Many drugs ha e been tried as treatment for HANE, for example adrenaline, antihistamine, chlorpromazine, corticosteroids and oestrogen, all without effect. After the introduction of methyl testosterone therapy in 1959 it was suggested that testosterone might counteract attacks of oedema by antagonizing the effect of histamine (52). Hamilton and Montagna (20) found increased thickness of collagenous fibres in the skin of ovariectomized hamsters during testosterone propionate treatment. It was suggested that testosterone would reduce the pathologically increased vascular permeability in HANE. Spaulding (52) successfully treated four members of a family with methyl testosterone in a dose of 10 mg to 40 mg daily A good therapeutic effect has later been reported with this therapy (14 25 42). The patient reported by Korsan-Bengtsen et al. (25) is case B 1. in this paper who before initiation of testosterone therapy was hospitalized for long periods because of his severe symptoms of HANE. Now—after years of treatment—he has had only few abortive ttacks and it has been possible to reduce the dose from 30 to 10 mg daily A few side-effects ha e been reported during methyl testosterone therapy in HANE, such as acne, gynaecomastia and facial hirsutism (57). Our patient has not noticed any such side-effects.

Epsilonaminocaproic acid (EACA) was first tried on a patient with HANE in 1960 by Landerman et al. but without any obvious effect (27). The drug was given intravenously during a few attacks of oedema and abdominal pain. Nilsson et al. (40) reported a case who during years had taken EACA immediately at the first sign o symptoms in a total amount arying between 7–133 g on each occasion. In this way as a rule he had been able to control the attacks. In association with 79 prodromes he had, up to 1966 taken EACA in an amount of 5 131 g. H noticed no side-reactions at all and liver function tests were normal. Lundh et al. (37) published a case of HANE which was treated with EACA during six months. He had no attacks during this period The dose of EACA was 6 g five times daily The authors stress that EACA must be given prophylactically and continuously in adequate doses. They noticed some side-effects such as nasal stiffness, slight dizziness and dry ejaculations, leading in this case to a prostato-vesiculitis. After changing the therapy to trans-4 (aminomethyl) cyclohexane carboxylic acid (AMCA) the ejaculations became normal and the prostato- vesiculitis disappeared. So did the nasal stiffness and dizziness. The patient took AMCA 5 g daily for eight months, during which time he had no symptoms of HANE and no side-effects at all. The patient is still symptom-free after now four years of treatment. Jahlin et al. (23) published two cases, a mother and her daughter who were treated with EACA and AMCA. During this therapy they developed more frequent and more severe symptoms. In a double-blind trial with EACA Champion and Lachmann could partially control the symptoms of HANE in two brothers (11) After that trial they changed the therapy to AMCA w th success. Two more HANE patients were treated with AMCA with excellent effect.

In this paper we have presented three men with HANE who were treated with EACA. Case A 1. has taken the drug both continuously for a couple of months and discontinuously immediately at the first sign of symptoms. It is now clear that he only needs intermittent EACA therapy During the last years he has taken the drug twice a month in a dose of 6–30 g daily during one or two days. This regimen has entirely changed his earlier so disabled life H only had two attacks of abdominal symptoms during the last year on both occasions EACA was administered too late after the ppearance of the prodromal symptoms. No side-effects have appeared after the 4 years of EACA treatment. Case B 1 was treated with

EACA during two periods for 5 and 7 weeks respectively with partial success. The therapy was stopped when he developed extensive muscle necrosis. The cause of this complication is not known but some possibilities were discussed by Korsan Bengtsen et al. (25) In case A 18 treatment with EACA was tried but had to be discontinued owing to gastrointestinal side-effects.

There are only a few reports of long-term treatment with EACA. No serious side-effects have been noticed except for the extensive muscle necrosis discussed above (25 47 50). The side effects of AMCA are less frequent but are of the same type as in EACA i.e. nausea, diarrhoea and orthostatic reactions (3).

EACA inhibits the activation of plasminogen to plasmin and is thus a strong inhibitor of fibrinolysis (1 2). In higher concentration EACA *inhibits trypsin, plasmin pepsin and other proteolytic enzymes* (1 2). It may inhibit the activation of $C\bar{1}$ (13 54). On the other hand Back and Steger found that EACA moderately inhibited the kinin-releasing activity of trypsin and kallikrein (7). AMCA has a higher antifibrinolytic activity than EACA and its effect is more protracted (3).

EACA and AMCA may interfere in several enzyme reactions, which may interact with each other. The result of this therapy on the HANE symptoms, therefore may vary. To further clarify the effect of antifibrinolytic therapy in HANE a double-blind trial with AMCA on five patients with HANE has been started, the results of which will be reported later (10).

Successful treatment of a few patients with HANE with plasma or blood transfusions has been reported (23 43) with measurable increase in inhibitor function and a decrease of free esterase activity in serum (43). It was proposed that the fresh plasma therapy might be used for *potentially life threatening or painful episodes of* HANE until an effective purified form of the $C\bar{1}$ INH becomes available.

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INFLUENCE OF PROBENECID ON GENTAMYCIN PHARMACOKINETICS

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Abstract. A previous study showed that gentamycin apparently is secreted by the renal tubular cells. T investigate whether this occurred via the same transport mechanism as the penicillins, probenecid has been given. No evidence for reduced gentamycin excretion has been found after this treatment, hich means that this compound must be transported via another system.

Recently a reduced excretion ratio of gentamycin (gentamycin clearance to creatinine clearance) was reported in patients with impaired renal function due to chronic pyelonephritis (1). For this, a calculation model developed by Fisher et al. (2) was employed. This lowered excretion ratio could be the result of among other factors, a reduced tubular secretion of gentamycin as discussed in the previous report. To elucidate this question, probenecid blockage of a specific tubular transport system has been performed to investigate whether gentamycin is possibly secreted by this system.

MATERIAL AND METHODS

Patients

Five patients were studied. Some clinical and laboratory data are presented in Table I. Two patients (SS and TG) had renal function within the normal range as evidenced by endogenous creatinine and inulin clearance. The renal function impairment in the three other patients was significant; in one (AR) the creatinine clearance was 13 ml/min and the inulin clearance below 10 ml/min.

Renal function studies

The renal function (GFR) was determined as inulin and creatinine clearance simultaneously ith the excretion studies of gentamycin.

Plan of study

On the first day the gentamycin xcretion was studied before and after one dose of probenecid. The second examination was made after three day of continuous probenecid treatment.

On both days of examination the patients rece ed 60 mg of gentamycin i.m. No other antibacterial agent was administered simultaneously

Two and half hours after gentamycin medication on the first day 0.5 g probenecid as gi en orally. Subsequently 0.5 g probenecid was administered twice for three days before the renewed excretion stud)

Sample collection

Details of sampling were as reported previously (1 cept that blood and urine samples were collected every half hour for four hours starting one hour after the gentamycin injection on the first day and for hour period on the second day of the investigation.

Gentamycin assay and calculations

Quantitation of gentamycin and calculations were carried out as described previously (1).

RESULTS

As will be seen from Table II, the excretion ratio of gentamycin is significantly below 1 in patients with reduced renal function, which is in accordance with previous results (1) however the data are few and difficult to evaluate. In two of the patients (SL, SS) the serum half-life remained unchanged after three days of probenecid, in two others (AR, KT) there was a *reduced* gentamycin half-life.

The study showed that gentamycin excretion was unaffected by probenecid. From the excretion curves in Fig. 1 one ppreciates that there was little difference in slope before and after medication on the first day and after probenecid had been taken for 70 hours. The regression of the curves is indicated in Table III. There was no significant difference in the regression slopes for

Table I. *Clinical and laboratory data of 5 patients in whom gentamycin excretion was studied before and after probenecid medication*

Pat.	Sex	Age (y.)	Diagnosis	Day of study	Mean clearances: Creatinine (ml/min)	Mean clearances: Inulin (ml/min)	Serum creatinine (g/100 ml)
SL	♀	28	Collagenosis	1	68	46	1.3
				2	40	37	1.6
KT	♀	30	Chronic glomerulo-nephritis	1	45	39	1.8
				2	48	37	2.0
AR	♀	56	Chronic pyelo-nephritis	1	16	21	3.7
				2	16	16	3.9
SS	♂	16	Nephrotic syndrome	1	104	86	0.9
				2	104	94	0.9
TG	♂	47	Recurrent urolithiasis	1	103	88	0.8

Table II. *Serum half-life and excretion of gentamycin in 5 patients studied before and after probenecid medication*

Pat.	Time of study[a]	Regression of excretion curve $y =$	Mean creatinine clearance (ml/min)	Mean inulin clearance[b] (ml/min)	Corrected gentamycin clearance (ml/min)	Excretion ratio related to: Creatinine clearance	Excretion ratio related to: Inulin clearance	Serum half-lif of gentamycin (h)
SL	BP	0.8241–0.0983x	69.0	46.0	20.6	0.30	0.45	3.0
	AP	0.8689–0.0931x	67.2	46.5	14.3	0.21	0.31	3.2
	Mean				17.5	0.26	0.51	2.1
	AAP	1.0962–0.1444x	39.8	37.3	20.0	0.51	0.56	2.1
	BP	0.9909–0.1117x	50.0	33.7	27.2	0.54	0.80	2.3
	AP	0.8839–0.1054x	40.7	38.8	28.9	0.72	0.74	2.7
	Mean				28.0	0.63	0.77	2.5
	AAP	1.1596–0.1912x	44.2	34.4	11.8	0.27	0.35	1.6
AR	BP	0.9608–0.0907x	21.3	18.0	14.6	0.69	0.81	3.3
	AP	0.7725–0.0242x	17.2	18.5	4.8	0.28	0.29	11.8
	Mean				9.7	0.49	0.55	7.6
	AAP	0.9693–0.1204x	15.8	15.5	8.6	0.55	0.55	2.2
SS	BP	1.2909–0.2975x	101.3	79.0	57.6	0.56	0.72	1.1
	AP	1.1743–0.2414x	102.7	89.1	43.7	0.43	0.50	1.2
	Mean				50.6	0.49	0.61	1.2
	AAP	1.3596–0.2447x	104.3	93.7	58.8	0.57	0.63	1.2
TG	BP	0.7136–0.2343	95.7	85.3	88.9	0.91	1.05	1.3
	AP	0.9463–0.2298x	98.7	85.8	75.8	0.77	0.88	1.3
	Mean				82.2	0.84	0.96	1.3

[a] On the first day the means of three successive half-hour collection periods were assayed both before (BP), and after (AP) 0.5 g of probenecid orally. AAP is the mean of four (in SL and AR) or three (in KT and SS) successive 30 min collection periods after 0.5 g probenecid had been given twice daily for 72 h.

[b] Lacking simultaneous analyses of creatinine and inulin, the mean clearances of six immediately preceding 30 min intervals were used for the calculation of gentamycin excretion for the two final periods on the first day. Similarly, for calculation of the fourth period in AAP the mean clearances for three preceding periods were used. The clearance means indicated in Table I are the true means of six or three collection periods, respectively, on the first or second day of study.

t-Tests failing to demonstrate a significant difference between serum level regression slopes for each of the two periods on the first day, means could be calculated.

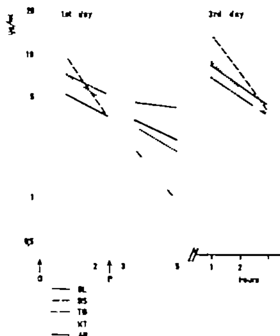


Fig. 1. Serum concentrations ($\mu g/ml$) of gentamycin in 5 patients related to hours after injection (*G*). The curves covering the intervals 1–2 ½ h are from before probenecid intake and the curves from 3 ½–5 h subsequent to probenecid intake (*P*). The curves have been calculated as regressions by the least squares method. The time indicates hours after the last dose of gentamycin. The findings from the study starting 48 h after the first dose of antibiotic are indicated to the right.

the three study periods with or without probenecid in the same patient. The serum half-lives for gentamycin and the excretion ratio remained within the same range (Table II)

Table III. *Results of t-testing for the similarity between regression coefficients according to the formula*

$$t = \frac{b_1 - b_2}{s_d}$$

The b_1 and b_2 are the regression coefficients compared, derivable from column three from the left in Table II, and s_d is measure for the scatter according to Weber

Pat.	BP vs. AP[a]	BP vs. AAP[a]	AP vs. AAP[a]
SL	0.04	0.23[b]	0.31
KT	0.03	0.36	0.39
AR	0.62	0.21[b]	0.08
SS	0.02	0.06	0.003
TG	0.004	NA	NA

[a] See Table II.
[b] Figures for which $f = 3$ (degrees of freedom) apply; others have $f = 2$. ($t(0.01; 2) = 9.92$ and $t(0.01; 3) = 5.84$).
NA Not applicable. (The patient could not be studied for the second day sequence.)

As a check, the current findings were plotted together with the data of the previous study (1) (Figs. 2 and 3), the relationships in which were substantiated.

DISCUSSION

Our previous study demonstrated an apparent secretion of gentamycin in patients with reduced renal function due to chronic pyelonephritis (1). The excretion ratio of gentamycin (the ratio between the gentamycin clearance and creatinine clearance) was significantly depressed in patients with reduced renal function. This could indicate that the secretion which normally took place was depressed in patients with tubular damage. If

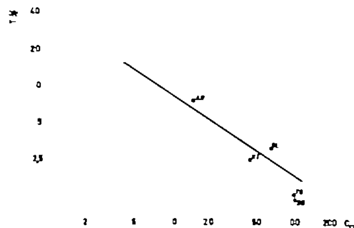


Fig. 2. Relationship between serum half life ($T½$) of gentamycin and the mean endogenous creatinine clearance (C_{cr}) during the first day of study. ○ the patients in this study; ● the patients from the previous investigation (1). The regression is $y = 1.618 - 0.668x$ with and y the log_e to the C_{cr} and $T½$ respectively. The correlation is significant ($p < 0.001$).

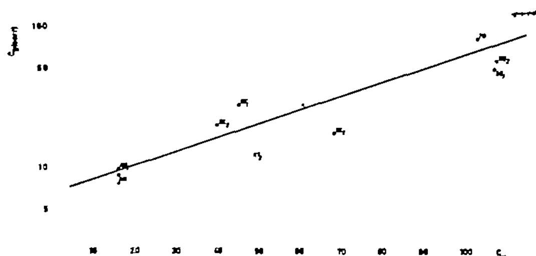


Fig. 3. Relationship between the corrected gentamycin clearance, C_g(corr), and endogenous creatinine clearance (C_{cr}). Symbols as in Fig. 2. The observations marked by 1 are the mean of the first day and 2 the mean of the second day of study (i.e. starting 48 h after the first dose of gentamycin). The line of regression follows the equation $y = 0.828 + 0.010x$, where x is C_{cr} and y is the $\log_{10}$ to C_g(corr). The correlation is significant ($p < 0.001$).

secretion occurred, it would be of interest to study whether this could be blocked by probenecid, in which case gentamycin would be transported via the same active transport mechanism as, for example, the penicillins.

Present findings failed to demonstrate such a mechanism. This could be due to differences in affinity for the tubular carrier but is also a question of the concentration of probenecid since competition of the transport system obeys the mass law. This circumstance was taken into consideration by treating the patients with probenecid for three days before gentamycin excretion was examined the second time. The excretion ratio was never significantly depressed. Although these findings still do not contradict our hypothesis of tubular secretion of gentamycin, the probenecid treatment would have effectively blocked the transport of penicillin and related compounds.

Two of the patients examined had normal renal function. In patient TG (Fig. 3) a high excretion ratio was found, as expected for normal functions (1). In the other (SS) the ratio, although in the higher range, was lower than expected. This patient had nephrotic syndrome. It is thought that the finding may be caused by binding of gentamycin to albumen which is passed in the urine in considerably increased amounts.

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FATTY LIVER IN DIABETES

A Cytological Study

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Abstract. Cytological liver specimens from 100 diabetics have been stained by the May-Grünwald-Giemsa method, and with oil red O. Satisfactory smears were obtained in 91 cases. Fatty liver was found in 48% and vacuolated nuclei in 57%. A close correlation was found between obesity and fatty liver and some correlation between the serum triglyceride level and fatty liver. The serum cholesterol level did not correlate with the incidence of fatty liver. This was commoner in maturity-onset diabetes than in young insulin-treated diabetics. Nuclear vacuolation was commoner in fatty-liver than in normal liver cells. Application of the fine-needle aspiration biopsy technique, together with staining of the smears with oil red O, was found to be a useful method for study of the incidence of fatty liver.

A common finding in diabetic patients is that of fatty liver. In most series of histological studies an incidence of approximately 50% or more has been found (6). Another phenomenon frequently observable in histological specimens taken from diabetic livers is nuclear vacuolation (16, 18). In diabetes mellitus the lipid metabolism is disturbed in many ways (2, 4, 9, 10). Consequently little surprise can be felt at the occurrence of liver steatosis, but it is not clear why some diabetics develop a fatty liver while others do not.

In this study an endeavour has been made to correlate liver steatosis in diabetics with the blood glucose and plasma lipid levels of the patients, with attention also being paid to the weight of the patients. The histological punch needle biopsy is not free from risk (14). Recently it has been shown that the diagnosis of fatty liver can be established cytologically (14, 17, 19) by the study of smears obtained by means of very thin needles (7). This method was preferred in the present study by reason of the almost negligible risk (14) even if localization of the fatty infiltration in the liver lobuli is impracticable. The lipid composition of the small liver fragments obtained with thin needles can be studied biochemically by the application of advanced techniques (11). In the present study a simple cytochemical method of fat staining was applied.

MATERIAL AND METHODS

Cytological liver specimens were obtained from 100 diabetics by the use of Franzén instrument (7). About one third of the biopsies were performed in the Out-patients Department, and two thirds in the wards. As a rule the site of the puncture was at the 9th intercostal space in the mid-axillary line, but some large livers were punctured by the abdominal approach. Nine cases with unsatisfactory smears were excluded. Thus the series comprised 91 patients, 57 females and 34 males. The average age was 60 years, ranging from 20 to 83 years. The disease averaged 6.6 years in duration. Most of the patients were treated with oral antidiabetic drugs and/or diet, while 4 were treated with insulin. Control of the diabetes was considered good if the fasting blood glucose level was generally below 1.3 g/l and the urine glucose less than 20 g/24 h, satisfactory if the blood glucose level was below 2 g/l and urine glucose less than 40 g/24 h. Control was considered poor if the values exceeded those mentioned. None of the patients was in a decompensated (ketoacidotic) state of diabetes, and none had consumed alcohol during the 24 hours immediately preceding the biopsy. Known alcoholics were all excluded from the series. In every case, records were made of the fasting blood glucose, plasma total lipid, triglyceride (TG) and cholesterol levels.

Some of the air-dried smears were stained by the application of the May-Grünwald-Giemsa method (MGG). Others were stained for the demonstration of fat by means of oil red O, with a modification of the procedure recommended by Lillie (13). The smears were fixed for 30 sec in cold solution of formalin in alcohol (1 part

Table I *Occurrence of fatty liver and of nuclear vacuolation of liver cells in diabetes*

Grade of steatosis	No. of cases	Nuclear vacuolation (and %)	Mean age (y)
0	47	14 (30)	57
1	18	12 (66)	63
2	18	18 (100)	65
3	8	8 (100)	67

Table II *Correlation between the control of the diabetes and the grade of liver steatosis*

Control of diabetes	Grade of steatosis (n and %) 0	1	2	3	Total with steatosis (n and %)
Good	23 (51)	8 (18)	9 (20)	5 (11)	22 (49)
Satisfactory	12 (46)	7 (27)	5 (14)	2 (8)	14 (54)
Poor	12 (60)	3 (15)	4 (20)	1 (5)	8 (40)

of 40% formalin to 9 parts of ethanol). The staining solution was prepared by mixing equal parts of saturated solution of oil red O in isopropanol and 50% solution of isopropanol in water. The incubation time was 5 min. After rinsing in tap water and counterstaining with haemalum, the smears were covered with Apathy gum syrup and cover slip. The person making microscopic study of the stained smears had no data available in regard to the patients.

The degree of steatosis was classified as follows: Grade 0 – no fat vacuoles, or small vacuoles in less than 20% of the cells. Grade 1 – small fat vacuoles in less than 90% of the cells. Grade 2 – large or small vacuoles in most liver cells, but fair amount of normal cells distinguishable. Grade 3 – large fat vacuoles in all or almost all cells, with at least some cells completely filled with fat, displacing the nucleus to the periphery (signet ring forms). Nuclear vacuoles were recorded if found.

The overweight of the patients was calculated as a percentage in accordance with the normal weight tables prepared by Finnish insurance companies. The results were tested statistically by the χ^2 test.

RESULTS

The fatty vacuolation of liver cells was easily recognizable in MGG-stained smears if these contained thin layers of small liver fragments (Figs. 1 and 2) but larger fragments consisting of several cell layers were stained too dark for evaluation. In doubtful cases staining with oil red O proved to be extremely useful (Figs. 3 and 4). This method made fatty infiltration discernible in both thin smears and large fragments.

No steatosis was apparent in the liver specimens from 47 of the 91 diabetics studied, whereas 44 (48%) had fatty liver (Table I). The commonest types of steatosis were grades 1 and 2. Nuclear vacuolation was observable in all subjects with grades 2 and 3 of steatosis, in about two out of three with grade 1 but in no more than 30% of the cases with no cytological evidence of fatty liver.

It seemed that the degree of steatosis increased with advancing age. Statistically the correlation was almost significant ($p < 0.05$). However no correlation was observable between steatosis and the duration of diabetes. For example, out of 14 cases with recently discovered diabetes, only four had normal liver cells, while four had grade 1, five grade 2, and one grade 3 of steatosis.

The occurrence of fatty liver was not more frequent in poorly controlled disease than in cases under good or satisfactory control (Table II). Fatty liver was much less frequent in patients treated with insulin than in those treated with oral drugs and/or diet. In the latter group steatosis was noted in 63% of the cases, whereas only 4 of the 24 (17%) patients treated with insulin had fatty liver.

Fatty liver was found much more frequently in obese patients than in other diabetics (Fig. 5). The correlation between liver steatosis and overweight was highly significant ($p < 0.0005$). In the normal or underweight group only 3 of 32 patients were found to have fatty liver, whereas 75% of the patients in the severely overweight group had steatosis of the liver. Most of the grade 2 and grade 3 steatosis cases were found in

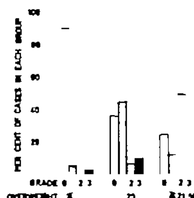


Fig 5 The incidence and grade of steatosis in three overweight groups.

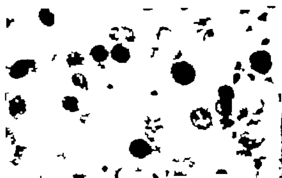








Fig. 1 Normal liver cells. MGG, 500.

Fig. 2. Fatty liver MGG, 500.

Fig. 3 Normal liver cells. Oil red O, 500.

Fig. 4 Fatty liver Oil red O 500.

the latter group. The serum TG values were somewhat higher in the overweight groups than in the normal–underweight group although the difference was not significant. A tendency to gain weight with increasing age was apparent. The median age was 57 years in the normal–underweight group, 63 years in the 1–20% overweight group, and 65 years in the more than 20% overweight group.

No correlation was found between serum cholesterol level and liver steatosis. Nevertheless high TG values were commoner in patients with fatty liver than in those with no signs of steatosis. In the steatosis groups some very high TG values (above 6 g/l) were recorded. If these five cases are excluded, together with the five cases with the lowest TG values in the same groups, the following average values are obtained: 1.6 g/l in the non-steatosis group, and 2.0 g/l in the steatosis groups. The difference is significant ($p < 0.01$). No correlation was found between the weight of the patients and the TG levels.

DISCUSSION

Fatty liver means a visible accumulation of lipids in the liver cells (2, 12). It may develop in four ways (6): 1) by the increased transport of lipids and fatty acids to the liver, 2) by enhanced lipid synthesis, 3) by reduced lipid oxidation in the liver and 4) by reduced lipid removal from the liver. Increased lysis of fatty tissues, resulting in an increased inflow into the liver, is probably the most important mechanism in diabetes (5). A disturbed lipoprotein synthesis, inducing the reduced removal of lipids from the liver, is a contributory factor (2, 10). Bengmark (1) has noted the accumulation of fat in the liver almost immediately after partial hepatectomy. This immediate steatosis was probably attributable to reduction in the liver mass. The main increase occurred in the TG fraction, but cholesterol and phospholipids also increased. As a rule fatty liver results from the accumulation of triglycerides (2). Abnormal glucose tolerance is found in connexion with genetically determined lipoprotein disturbance only when the TG level is elevated (8). Increased lipolysis in diabetes is attributable either to insulin deficiency or to obesity. In juvenile diabetes a true insulin deficiency is present, whereas the plasma insulin level may be high in the maturity-onset type, particularly in obese patients, and most of them are obese (4, 15). In these a relative deficiency is caused by diminished sensitivity to insulin. Since the metabolic disturbance in this type of diabetes is different from that in the juvenile type, it is not surprising that the incidence of liver steatosis is different as well.

In 1970 Creutzfeldt et al. (6) reviewed 16 histological investigations on the frequency of fatty liver in diabetes. The incidence ranged from 21 to 78% and the average incidence of fatty liver calculated in respect of the whole accumulated material, was 50%. The result of our cytological study completely corresponded with this finding, as we observed fatty liver in 48% of our diabetic subjects. Beringer and Thaler (3) have studied histological liver biopsies obtained from 465 diabetics, and recorded fatty liver in two thirds of the cases. In a small series studied by Takeuchi et al. (18) the incidence was 55%.

Fatty liver is not a common finding in juvenile diabetes (3, 6). In our series only 17% of young diabetics treated with insulin had fatty liver. However, 63% of the patients suffering from maturity-onset diabetes had fatty liver in our series examined by cytological methods. This proportion is in close agreement with the results obtained in histological biopsy studies (3, 6). We found a close correlation between the degree of obesity and the incidence of fatty liver, and also a significant correlation between hypertriglyceridemia and fatty liver. Most of the patients suffering from maturity-onset diabetes are obese, and we agree with Creutzfeldt et al. (6) that obesity per se may be a more important etiological factor in the development of fatty liver than is diabetes.

The true incidence of fatty liver cannot be studied by means of autopsies, since the results are influenced by the terminal phase of the disease. In most, if not all, histological biopsy studies the series of patients has been selected in various ways. As far as we know, this report represents the first incidence study of fatty liver in diabetics by the application of a cytological technique. Our series has to be considered as a selected one, as two thirds of the patients were hospitalized for one reason or another. The results we arrived at are in close agreement with those derived in previous studies by means of histological methods.

It may thus be concluded that the cytological liver biopsy technique supplemented by a simple cytochemical method, is appropriate for studies of this type. Cytological aspiration biopsy is a minor procedure, applicable for the study of truly un selected series of diabetic ambulatory patients and healthy people both as such with normal weight, and obese people Such a study should be made for clarification of whether fatty liver is induced by diabetes, by obesity or both factors together

The occurrence of fatty liver in diabetes is a sign of disturbed fat metabolism and may thus play a role in the progress of the diabetes, how ever the prognosis of the liver disease itself is good. As distinct from the fatty liver of alcoholics, diabetic fatty liver does not generally proceed to cirrhosis (6, 12)

Vacuolated liver cell nuclei are common in diabetes, but are not specific for this disease (6) We found them in 57% of our diabetic subjects, most frequently in fatty livers. It is known that the vacuoles contain glycogen (16) but their significance is unknown In all probability they do not influence the liver function or the prognosis.

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LONG-TERM PROGNOSIS AFTER VENTRICULAR FIBRILLATION IN ACUTE MYOCARDIAL INFARCTION

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Abstract. During the period 1965–1969 a total of 87 patients with acute myocardial infarction (AMI) have been discharged from hospital alive after recovering from one or more episodes of ventricular fibrillation (VF). The average age was 59 years (39–82). Up to the time of follow-up the group had been observed for 6–62 months. At the follow-up 47 of the survivors were alive and the average age was significantly lower than that of the non-survivors. The prognosis was not found to be significantly better in the cases of "primary" as opposed to "complicating" VF. The actual time of the first VF had no significant influence on the survival rate. Normal heart volume upon discharge was found to be a favourable factor in prognosis. After convalescence 16 of the 44 patients in employment prior to the onset of disease returned to work. Other factors influencing return to work are discussed.

Few papers have hitherto been published concerning follow-up studies of patients discharged alive after ventricular fibrillation (VF) during acute myocardial infarction (AMI). The materials have been relatively small and the time of follow-up has been short, except for one substantial material (6). In the present paper 87 patients who survived myocardial infarction in spite of VF episodes are followed from 6 to 62 months.

MATERIAL

During the period 1965–1969 the Medical Intensive Care Unit in the Central Hospital of Tampere treated a total of 63 male and 24 female AMI patients who could be discharged alive after one or more episodes of VF. The average age of the patients was 59 years (range 39–82). In addition to their coronary disease two patients were diabetics, three suffered from intermittent claudication, two had sustained stroke attack and eight had high BP previous to their attack. In all cases the diagnosis of AMI was based upon WHO criteria (12). Only such cases were accepted for the present material in which VF was registered in the cardioscope and/or in the ECG as the immediate cause of cardiac arrest. VF was taken as "primary" if the attack was not preceded by congestive heart failure or hypotonia, otherwise VF was taken to be "complicated" by cardiac incompensation or hypotonia, i.e. systolic pressure under 100 mmHg (3, 8).

Treatment was in accordance with standards presented in earlier papers from this hospital (4, 5, 9). Most of the patients were discharged within 3 or 4 weeks after the onset. Anticoagulant treatment was continued at home wherever it could be properly supervised. Anti-arrhythmic drugs were continued after discharge in 25 cases in which the tendency to dysrhythmia appeared to persist.

Follow-up examinations were made in March–June 1970. On 30 June 47 patients of the original material were alive; 45 attended the clinical examination. In the history special attention was directed to physical and mental capacity. The examinations comprised clinical status, chest X-ray, ECG and prothrombin time. Two patients who had moved to other areas gave the required information by post, enclosing X-ray reports and ECGs. Previous to 30 June 1970 40 patients had died.

RESULTS

The average age of the 47 survivors calculated at the onset of AMI was 56.8 years. That of the 40 who had died prior to the follow-up was 61.8 years. The difference is statistically significant ($p < 0.01$).

Age and sex distribution of the material and the survival pattern according to age are shown in Table I. In each of the age groups the proportion of males was greater. As age increased, the relative proportion of survivors diminished almost significantly ($X^2 = 7.91$, df $= 2$, $p < 0.02$).

Primary VF was diagnosed in 55 cases as the cause of cardiac arrest: 33 (60%) were alive at the follow-up (Table II). VF had started outside hospital (at home, on the street, in the ambu-

Table I *Age and sex distribution of patients discharged alive (figures in parentheses denote patients surviving to follow-up)*

	Age (y)				
	35–44	45–54	55–64	65–	Total
Male	10	16	17	20	63
Female	—	5	7	12	24
	10 (7)	21 (15)	24 (12)	32 (13)	87 (47)

Table II *Type of VF and location of patient at time of episode*

	Primary		Complicating	
	Total	Alive	Total	Alive
Outside hospital	16	4	1	—
Emergency Department	16	12	2	1
Intensive Care Unit	11	11	19	8
Elsewhere in hospital	12	6	10	5
	55	33	32	14

lance, etc.) in 17 cases. Nine of these patients sustained serious brain damage. They had to be transferred to hospitals for chronic patients. All them died prior to the follow-up. Five lived than 3 months, the survival of the remainder ranged from 3 to 37 months. The average age of the cases with cerebral lesion was 67 years, only one was under 64. If these nine cases are omitted, 72% of the patients with primary VF were alive at the follow-up. All the subjects who suffered primary VF in the Intensive Care Unit were still alive at the follow-up.

Complicating VF was stated as cause of cardiac arrest in 32 cases, of whom 14 (44%) were alive at the end of follow-up (Table II). Nineteen patients had suffered VF in the Intensive Care Unit, most of them had pulmonary edema. Eight were still alive at the follow-up. During 1965–69 only a bare half of all AMI cases could be admitted to the Intensive Care Unit (7). Thus ten of the complicating VF episodes started in general wards.

The survival percentage of those suffering primary VF is not significantly higher than that of the complicating cases ($X = 1.325$, $df = 1$, $p > 0.05$) when the cases with cerebral damage are included.

The first episode of VF occurred within 24 hours after the onset of AMI symptoms in 54 cases (Table III). At the follow-up 30 of these patients (56%) were alive. Among non-survivors were all nine cerebral damage patients. VF occurred after 24 hours in 33 cases, 17 of whom (52%) survived to the follow-up. The survival percentages do not differ significantly ($X = 0.02$, $df = 1$, $p > 0.05$).

Heart volume at time of discharge was normal in 43 cases, 34 (79%) of whom attended the follow-up. Enlargement of the heart (≥ 500 ml/m²) was observed in 39, 13 of whom lived to the follow-up (33%). The difference in survival rate is highly significant ($p < 0.001$). In five cases heart volume was not measured.

The duration of follow-up is given in Table IV. Eighteen patients died within 6 months after the onset of AMI. Two had lived more than 5 years, five more than 4 years and the largest group reviewed had lived 1–2 years.

The clinical examination of the survivors revealed that none of the 47 patients suffered any remarkable loss of memory. Eighteen complained of mild absent-mindedness. Two had suffered a further AMI during the follow-up period and had recovered. In only two cases did clinical examination reveal actual arrhythmias, atrial fibrillation

Table III. *Type of VF and time of occurrence calculated from commencement of myocardial infarction*

	< 24 h		> 24 h	
	Total	Alive	Total	Alive
Primary	44	25	11	8
Complicating	10	5	22	9

Table IV *Observation span of patients at time of death or up to 30 June 1970*

	Months						
	6	6–12	12–24	24–36	36–48	48–60	60
Alive	—	7	20	7	6	5	2
Dead	18	8	9	4	1	—	—
	18	15	29	11	7	5	

Table V *Return to work of patients eligible for work prior to myocardial infarction (survivors in parentheses)*

	Primary VF	Complicating VF	Total
Returned to former employment	12	1	13 (13)
Took up lighter work	2	1	3 (2)
Retired	17	11	28 (20)
	31	13	44 (35)

in one and ventricular ectopic beats in one case. Twenty-eight patients were on digitalis treatment, manifest congestive failure was noted in three patients. Twenty were still receiving anticoagulants and 12 were under continuous anti-arrhythmic therapy. Two were able to do without any medication.

Prior to their disease leading to VF 44 patients had been working, of whom 13 returned to their former occupations and 3 took up lighter work (Table V). Only one returned to heavy physical work. Twenty-eight patients retired, of whom 8 died prior to the follow-up. Previous to the onset of AMI 22 of them had been engaged in moderately heavy or heavy work. The average age of the 16 persons who returned to work was at the onset of AMI 47.7 years and of those retiring 57.9. The difference in age is statistically highly significant ($p < 0.001$).

Up to the end of June 1970 40 patients had died, of whom 32 had succumbed to their coronary disease according to death certificates. Fifteen patients had died in the Central Hospital of Tampere when readmitted, 14 in other hospitals and 11 at home. The diagnoses were confirmed by autopsies in 15 cases; 14 of those deaths were sudden. One had died of cerebral thrombosis, two of pneumonia, and in five cases information about the immediate cause of death could not be obtained.

DISCUSSION

During the period 1965–69 over 300 patients with AMI were successfully resuscitated in this hospital. About one third of them could eventually be discharged alive (4). The present material comprises only confirmed cases of VF.

A material of resuscitation of AMI patients from this hospital has previously been published, and 19 of them were followed up to 19 months (5, 9). Many post-discharge studies have comprised relatively small materials. Geddes et al. (2) discuss 50 cases, Stannard and Sloman (10) 20 cases and Lawrie (3) 53 patients who had survived VF following myocardial infarction and been discharged. The observation period in these studies ranged from 6 months to 4 $^1/_2$ years. In 1970 McNamee et al. (6) published a study of 160 VF cases followed up over a period averaging 15 months. The present group of 87 patients was followed up for an average of 18.4 months, the maximal observation time exceeding 5 years.

No age limit was applied in the resuscitation of AMI patients. Thus the age group over 65 years was the largest. In this group the mortality was high raising the mortality rate for the material as whole. In the Belfast study (6) the death rate was somewhat lower. In part the difference is due to the fact that nine of our patients sustained severe brain damage owing to delay in resuscitation. All of them died prior to the follow-up examination. On the other hand it should be noted that eight patients of the present series made excellent recoveries in spite of the fact that their VF commenced outside hospital. No mobile coronary care unit was available, but the normal ambulance service was efficient. The hospital staff were not issued with instructions to apply any four-minute limit after which no resuscitation measures should be started (1).

Prognosis has been found to be better in the AMI cases in which VF is associated with clinically mild disease (2, 6) or in other words, in which VF is primary (3, 10). In the present study primary VF occurred in 55 cases. Two out of three cases were alive at the follow-up, but their survival rate was not significantly better than that among the complicating cases. If the cases with brain lesion are omitted from the group suffering primary VF the remaining group is seen to have the better prognosis.

According to McNamee et al. (6) the prognosis is better if VF occurs early in the course of disease. In the present study no difference was observed between those in whom VF started within 24 hours after onset of AMI and those in whom VF commenced later. The incidence of primary VF was seen to be greater in the former

group. All the cases in whom VF started outside hospital were regarded as having primary VF. The inclusion of these patients—with nine cases of cerebral damage—raised the mortality rate for the group as a whole.

The prognosis was found to be highly significantly more favourable in cases having a normal heart volume at the time of discharge. This observation is in accordance with studies in which enlargement of the heart is found to be a disadvantageous factor in the prognosis of AMI (7, 11).

Only 16 of the 44 patients who had been working prior to their myocardial infarction returned to regular employment. Other studies report the majority of cases with primary VF as returning to work (3, 6, 10). In this study most of those who retired had been engaged in heavy occupations and were of relatively advanced age. Furthermore the present pension legislation in this country is apt to discourage patients at rehabilitation from attempts for a new and possibly less highly paid employment.

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NEUROPHYSIOLOGICAL STUDIES IN HEREDITARY AMYLOIDOSIS WITH POLYNEUROPATHY

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Abstract. Sixteen cases with histologically verified amyloidosis with polyneuropathy have been investigated electromyographically (EMG) and motor conduction velocity (MCV) has been recorded. Eleven cases were familial and five sporadic. Clinically the polyneuropathy was pronounced in nine cases, moderate in six, and slight in one case. The EMG changes correlated well with the clinical findings and with the duration of symptoms, and were more marked in the long-standing cases. The MCV was abnormal, but correlated less well with the clinical pattern. The denervation was most obvious in the distal parts of the lower extremities. EMG of the short toe extensor muscles was found to be an especially useful examination. The findings in familial and sporadic cases were similar. The same examinations were performed on nine members of one afflicted family. Examinations of biopsy specimens of the skin did not reveal amyloid deposits. In two of the nine cases no clinical or neurophysiological findings of peripheral polyneuropathy were revealed. In seven cases clinical symptoms and/or signs of polyneuropathy were observed. They were, however, discrete. Three of these seven cases showed neurophysiological changes. In four patients no conclusive neurophysiological abnormalities were found.

During the last decades a hereditary type of primary amyloidosis has been described, in which progressive polyneuropathy with onset in adult life is a prominent clinical feature. Hereditary primary amyloidosis with polyneuropathy was first reported by Andrade (4) from Portugal, and later on familial cases with a similar pattern of neuropathy have been described by authors in different countries (1 6, 9 12, 13). In those cases the neuropathy was most pronounced in the lower limbs. A somewhat different symptomatology was found in two families in the USA (15 16, 17). In the latter cases the neuropathic symptoms usually preponderated in the upper limbs. Often the only manifestation of the disease was a carpal tunnel syndrome.

Since 1965 some 40 cases of histologically verified primary amyloidosis with polyneuropathy have been diagnosed in the Umeå region in the northern part of Sweden. Most of these cases are familial. Some of them have been reported earlier (2, 3). Ten cases are, so far considered sporadic. A common feature in all the cases was a progressive peripheral polyneuropathy with both sensory and motor disturbances. In addition, signs of involvement of the autonomic nervous system were reported such as micturition difficulties, im potence orthostatic hypotension, and disturbed sweating. Gastrointestinal dysfunction with malabsorption, signs of heart affection, and opacities of the corpus vitreum were also observed. In all cases the symptoms of neuropathy started in the lower limbs. In the neurological examinations the symptoms were more pronounced in the legs.

We have investigated some of our cases in whom amyloidosis was histologically verified electromyographically (EMG) and have also deter mined the motor conduction velocities (MCV). Our purpose was to correlate the results from the neurophysiological examinations with the clinical stage as well as with the duration of the disease.

The genetic predisposition of this disease is said to be inherited as an autosomal dominant trait (4). W therefore examined some persons in the 50% risk zone in whom none or only discrete symptoms or signs of neuropathy had been found. These persons all had one parent with histologically erified amyloid polyneuropathy It

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Table I. *Neurophysiological findings in 16 cases of histologically verified primary amyloidosis with polyneuropathy*

Case	Sex	Age (y)	Duration of symptoms (y)	Clinical grading of neuropathy[a]		EMG[b] M. interosseus dors. I (hand)		EMG[b] M. tibialis ant.		EMG[b] M. extensor dig. ped. brevis		MCV[c] (m/sec) N. ulnaris		MCV[c] (m/sec) N. peroneus	
				Arms	Legs	Sin.	Dx.	Sin.	Dx.	Sin.	Dx.	Sin.	Dx.	Sin.	Dx.
A:6	♂	64	6	++	+++	2	2	2	2	2	3	46	54	32	TD
B:1	♀	60	4	+	+++	0	0	2	2	3	3	—	49	TD	TD
B:2	♀	68	5	+	++	2	2	3	3	3	3	37	32	TD	TD
B.3	♂	44	6	+	++	1	1	2	2	3	3	44	36	TD	TD
B:6	♂	59	8		++	2	2	2	2	3	3	38	35	TD	TD
B 7	♀	35	2	0	+	0	0	1	1	2	2	62	52	44	41
C.2	♂	35	6	++	+++	2	2	2	2	3	3	57	44	TD	TD
E.1	♀	64	9	++	++	2	2	2	2	3	3	42	47	TD	TD
E.2	♂	70	14	++	+++	2	2	3	3	3	3	30	37	TD	TD
F 1	♂	62	6	+	++	1	1	1	1	3	1	—	49	TD	29
G 1	♂	58	3	++	++		2	2	2	3	3	41	43	TD	TD
J	♂	38	2	+	+	1	1	1	1	3	3	52	47	35	36
K	♂	57	4	+	++	1	1	1	1	2	3	46	58	31	TD
L	♂	57	7	++	+++	2	2	2	2	3	3	41	29	TD	TD
M	♂	69	4	++	++	1	2	2	2	3	3	37	36	TD	TD
N	♂	61	14	++	+++	2	1	2	2	3	3	26	49	TD	TD

[a] 0 = no, + = slight, ++ = moderate, +++ = pronounced.
[b] Degree of denervation. 0 = no, 1 = slight, 2 = moderate, 3 = pronounced or total.
TD = total denervation. — = not performed.

was expected that the neurophysiological examination might contribute to an early diagnosis.

We also hoped to find out which one of the two neurophysiological methods is more reliable in early cases and, furthermore, which muscles should be examined in the first place. It is also of interest to know whether neurophysiological abnormalities can be revealed before clinical signs have appeared.

According to the literature, neurophysiological examinations in histologically verified cases of this type of primary amyloidosis are scanty. In earlier studies (5, 6, 8) the questions mentioned have not been discussed.

MATERIAL

The material consisted of 25 subjects, 15 men and 10 women. Twenty cases were familial and 5 were sporadic. The material was divided into two groups.

Group I consisted of 16 patients, 12 men and 4 women, with histologically verified amyloidosis (11). At the examination their ages ranged between 35 and 70 years, mean 58 years (Table I). In accordance with an earlier report by one of us (2), each family was indicated by a capital letter. In addition, every patient was given a number. The 11 familial cases reported here were marked A:6, B.1, 2, 3, 6, 7, C.2, E.1, 2, F:1 and G.1. Five patients were sporadic cases and were marked J, K, L, M and N. All the patients were considered to have primary amyloidosis—none of them had tumour, inflammatory disease or myelomatosis.

Group II included 9 persons, aged 28 to 51, mean 40, years, children of parents with amyloid polyneuropathy. In these 9 subjects, 3 men and 6 women, no amyloid was, however, found in biopsy specimens of the skin, and they had no or only discrete neurological symptoms and/or signs, as listed in Table II. The affected parent was the mother in 7 cases and the father in 2. Both parents belonged to family B in the earlier reported material (2) (Fig. 1). Seven cases in group II were examined twice with an interval of 15 months.

METHODS

Clinical evaluation

The diagnostic criteria indicative of polyneuropathy were grouped as follows.

Symptoms. Dysesthesia and paresthesia, dull pain, attacks of shooting pains, muscle cramps, and weakness in the distal parts of the extremities. A marked and troublesome feeling of coldness was also interpreted as manifestation of the disease.

Signs. Muscular atrophies, flaccid paresis with areflexia, and sensory disturbances. The sensory modalities tested were pain (pin-prick), light touch (piece of cotton and pressure-aesthesiometer), temperature (warm and cold

Table II. *Neurophysiological findings in 9 children of two patients with hereditary amyloid polyneuropathy. Clinically no or slight neuropathy, amyloidosis histologically not confirmed*

						EMG									
			Duration of symptoms	Clinical grading of neuropathy[a]		M. interosseus dors. I (hand)		M. tibialis ant.		M. extensor dig. ped. brevis		MCV (m/sec)			
												N. ulnaris		N. peroneus	
Case	Sex	Age (y.)	(y.)	Arms	Legs	Sin.	Dx.	Sin.	Dx.	Sin.	Dx.	Sin.	Dx.	Sin.	Dx.
II a	♂	51	—	0	+	0	0	0	0	0	0	45	47	46	43
						0	0	0	0	0	0	50	55	37	40
b	♀	49	5	+	++	0	0	0	0	0	0	54	64	47	45
						0	0	0	0	0	0	53	59	51	48
	♀	48	4	+	+	0	0	0	0	0	0	68	57	54	49
						0	0	0	0	0	0	59	55	47	47
d		44	—	+	+	0	0	1	0	1	1	65	53	45	45
						0	0	1	1	1	1	[illegible]	56	41	47
	♂	42	2	+	+	0	0	0	0	0	1	67	60	44	37
						0	0	0	0	0	1	67	60	46	39
f	♀	40	2	0	+	0	0	1	1	1	1	57	57	46	46
						0	0	1	1	1	1	60	55	50	45
g	♀	33	1	0	++	0	0	0	0	1	1	61	62	37	45
						0	0	0	0	1	1	48	50	44	45
h	♀	32	—	0	0	0	0	0	0	0	0	62	65	52	59
i	♂	23	—	0	0	0	0	0	0	0	0	61	60	52	45

[a] 0 = no, + = slight, ++ = moderate. [b] 0 = no, 1 = slight denervation.

water), passive movements, and vibration (tuning fork and biothesiometer).

The neuropathy was graded on the basis of severity in the following way:

Slight neuropathy (+). Characterized by attacks of pain, feeling of coldness, dysesthesia and paresthesia in the distal parts of the limbs. There was some reduction in superficial sensibility especially to pain and temperature, but no disturbances in proprioception were found. Slight distal weakness might have been present but no obvious muscular atrophy was noticed. The tendon reflexes were normal.

Moderate neuropathy (++). Involved persistent ache and attacks of shooting pains in the limbs. Dysesthesia and paresthesia were present in the proximal parts of the limbs. Superficial sensibility was lost or greatly reduced distal to the ankles and wrists. Some impairment of the vibration sense and of the sense of passive movements of the digits was observed. A moderate degree of muscular wasting and weakness was present in the distal parts of the limbs. The ankle jerks were absent and the other tendon reflexes reduced.

Pronounced neuropathy (+++). Irritative sensory phenomena were less conspicuous. Superficial sensibility was lost at least distally to the knees and elbows. The sense of passive movements and of vibration was lost or greatly reduced at the ankles and wrists. Pronounced muscular atrophy and distal paralysis were present. The ankle, knee, supinator, biceps and triceps jerks could not be elicited.

Electromyography (EMG)

The electromyographic investigation was performed with concentric needle electrodes. A DISA electromyograph (14A20) with frequency response of 10 Hz – 20 kHz was used. The amplified signals were fed to Tektronix 565 oscilloscope, which permitted simultaneous detailed study of the potentials and photography at fast sweep speeds. The needle electrode was inserted at random into the muscles under study (Tables I and II), and the activity was studied with the needle in three or four different positions. The activity was observed during rest (to detect denervation activity), during slight voluntary contraction (to study the amplitude and shape of individual potentials) and during maximal voluntary contraction (to study the interference pattern). The pathological findings were graded in the following way:

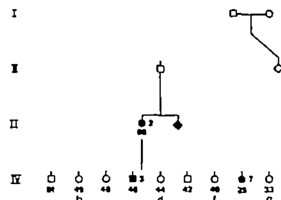
1. *Slight denervation*. A small number of fibrillations, positive sharp waves at rest, polyphasic potentials at slight voluntary contraction, occasional high voltage (5–10 mV) potentials at maximal voluntary contraction.

2. *Moderate denervation*. Fibrillations and positive sharp waves upon insertion of the needle and during relaxation, increased number of polyphasic potentials and/or high voltage potentials at moderate contraction; clear-cut decrease of the number of motor units activated during maximal voluntary contraction.

3. *Pronounced denervation*. Fibrillations and positive sharp waves upon insertion (in long-standing cases sometimes no denervation activity was recorded), high-voltage action potentials at slight activation, greatly decreased number (or total lack) of potentials at maximal effort.

Motor conduction velocity (MCV)

The conduction velocities were determined in the peroneal, ulnar and sometimes the median nerves, on both sides. The stimulation electrode was a DISA bipolar surface electrode (13K62). The recording electrode was of the



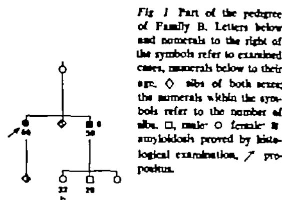


Fig. 1. Part of the pedigree of Family B. Letters below and numerals to the right of the symbols refer to examined cases, numerals below to their age. ◇ sibs of both sexes; the numerals within the symbols refer to the number of sibs. □, male; ○ female; ■ amyloidosis proved by histological examination. ↗ propositus.

same type, but at times a concentric needle electrode was used. A check was made that the action potentials of the muscles had the same appearance with regard to polarity and shape from both stimulation points of the nerve. The stimulator, DISA Mississippi, delivered rectangular pulses, the duration of which was set at 0.2 or 0.5 msec. Supramaximal shocks were always used.

The latencies were measured to the beginning of the muscle potential, i.e. the MCV of the fastest conducting fibres was always calculated. Under such circumstances the MCV in the upper extremities should not be less than 45 m/sec and that of the peroneal nerve not less than 40 m/sec. Marked differences (more than 10 m/sec) between two corresponding nerves of the same patient were considered abnormal, even if the lower MCV value exceeded the limit accepted as normal.

RESULTS

Group I

Clinical examination. The symptoms and signs of polyneuropathy appeared first and were more pronounced in the lower limbs in all cases (Table I). The manifestations were symmetrically scattered and more prominent in the distal parts of the extremities. According to our diagnostic criteria the neuropathy was pronounced in nine patients (A:6, B.1 ..., C..., E.2, G·1, L, M, and N). In the remaining seven cases it was moderate (B.3, 6, E.1, F.1, J and K) or slight (B.7).

Electromyography. The EMG changes were conspicuous in the distal parts of the lower extremities, even in an early stage of the disease (Table I). The degree of denervation, classified according to the principles stated under Methods, corresponded well to the degree of neuropathy as revealed by the clinical examination. The EMG pattern correlated well with the duration of symptoms and the degree of pathological findings.

Motor conduction velocity. The MCV was generally much below the normal limits in the affected cases (Table I). In most patients with pronounced clinical signs a complete denervation of the short toe extensors made it impossible to estimate the MCV of the peroneal nerves. In the remaining patients (A·6, F.1, J and K) the MCV of the peroneal nerves was prolonged, 29 to 36 m/sec. The MCV of the ulnar nerves was also prolonged, 26–39 m/sec. In some patients however (A·6, C.2, E.1, F.1, J, K, and N) the MCV of one or both ulnar nerves was normal in spite of clinical and electromyographic signs of neuropathy. On the other hand there were patients whose neuropathy from a clinical point of view was classified as slight or moderate, but in whom neurophysiological investigations revealed a neuropathy (B.3 and B·6) which was pronounced.

Group II

Age, duration of symptoms at the time of the first examination, clinical evaluation, and the findings of the neurophysiological investigations in both examinations (interval 15 months) are given in Table II.

Four patients (B:a, d, h, and i) had no complaints suggesting peripheral neuropathy. Five patients (B:b, c, e, f and g) had symptoms suggestive of neuropathy of 1 to 5 years duration. In seven cases (B:a–g) the clinical examination revealed slight to moderate signs of neuropathy. In most cases the symptoms and/or signs were discrete. Clinically the first and the second examination gave the same results.

The clinical findings were evaluated and correlated to the neurophysiological findings. The cases were grouped as follows:

1 Cases without clinical and neurophysiological signs of polyneuropathy 2 cases (B:h and B:f).

2 Cases with clinical signs of neuropathy but without neurophysiological abnormalities. 2 cases (B:b and B:c)

Case B:b

A 49-year-old woman, suffering since 5 years from coldness in both hands and feet. She also had attacks of pain and cramps in her lower legs. The ankle jerks were absent, there was some weakness of the toe extensors and impaired superficial sensibility in the distal parts of her legs. The EMG and MCV were normal in both examinations.

Case B:c

A 48-year-old woman, had noticed dysesthesia with burning and numbness in hands and feet during the last 4 years. She also had periods of persistent ache and short attacks of shooting pains below the knees. Examination revealed slight atrophy and weakness of the short toe extensors and reduced sensibility distally in her legs. The MCV were normal. At maximal voluntary contraction moderately reduced pattern of interference was observed. This, together with an unspecific tremor, was judged as suggestive of functional weakness in the absence of other signs of denervation.

3 Cases with clinical findings suggestive of neuropathy but without conclusive neurophysiological changes. 2 cases (B:a and B:e)

Case B:a

A 51-year-old man, denied having any symptoms indicative of polyneuropathy. At the age of 34 he had undergone thyroidectomy because of thyrotoxicosis. The examination revealed slight weakness in the extension of the left big toe. The perception of temperature and pain was somewhat impaired distally in the legs. Thus the clinical findings were scanty. The first neurophysiological examination was normal. In the second examination the MCV of the peroneal nerves were abnormally slow.

Case B

A 42-year-old man, had suffered from right-sided sciatic pain few years earlier. He complained of numbness of the ulnar parts of both hands. The hypothenar muscles were slightly atrophied and there was reduced ulnar sensibility bilaterally as well as reduced sensibility medially in the left foot and slight weakness of the dorsal extension of the left big toe. Neurophysiological examination revealed signs of neuropathy in the right leg only.

4 Cases with evident clinical and neurophysiological abnormalities: 3 cases (B:d, B:f and B:g).

The clinical examination of three women, 44, 40 and 33 years of age, revealed signs suggestive of neuropathy. Case B:d, however, denied any symptoms. In all three cases weakness of the short toe extensors and reduced superficial sensibility in the feet was observed. Case B:d had atrophy of the small muscles bilaterally in hands and feet. No ankle jerks could be elicited in case B:g. She also complained of pain in her toes and of weakness of her lower legs. Case B:f suffered from dysesthesia in the feet. Neurophysiological examinations revealed signs of peripheral neuropathy in these three cases. In the EMG signs of denervation were found in the short toe extensors and in cases B:d and B:f also in the anterior tibial muscles. Examples of abnormal potentials found in case B:f are shown in Fig. 2.

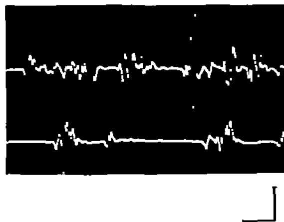


Fig. 2. Examples of pathological, polyphasic motor unit potentials in case B:f, group II. Calibration 20 msec, 1 mV.

In none of the nine cases in group II did EMG of the first dorsal interosseal muscle reveal any abnormalities, nor was the MCV of the ulnar nerve below the normal limit. At the first examination of case B:d the MCV of the ulnar nerves was 65 and 53 m/sec, a difference of 12 m/sec. In case B:g the MCV of the ulnar nerves fell between the first and the second examination from 61 to 48 m/sec and 6_ to 50 m/sec, respectively. The MCV was well above the lower limit in both cases. The differences are, however striking.

COMMENTS

Group I

The clinical studies of patients with histologically *verified* primary amyloidosis with polyneuropathy ha e indicated that the neuropathy was more prominent in the distal parts of the limbs (2, 3). The clinical signs of neuropathy were more pro-

nounced in the lower than in the upper limbs. The neurophysiological examination of the 16 patients presented in this study confirmed the clinical findings. Irrespective of the duration of their symptoms, EMG revealed marked changes in the short toe extensor and the anterior tibial muscles. The denervation made measurements of MCV in the lower limbs impossible in many cases. In addition to sensory disturbances in the distal parts of the legs, other early and typical manifestations of the disease were weakness, atrophy and EMG changes of the short toe extensor muscles.

Although both clinical examination and EMG revealed neuropathy of the upper limbs of all patients, the MCV was within normal limits in one or both ulnar nerves in seven cases. This is probably due to the fact that efferent fibres, capable of conducting impulses at a velocity considered normal, were still existing at the time of the examination. A significant difference between the two sides could, however, be established in some cases, indicating an incipient impairment of function.

In the large families in the USA (15, 16) the neuropathy in the upper limbs dominated. The manifestations seem to have been related to a carpal tunnel syndrome and not to general polyneuropathy. Most of the cases examined by neurophysiological methods (15) had a prolonged MCV of the median nerve, while that of the ulnar nerve usually was normal. In three cases, however, the MCV was reported to be more reduced in the lower than in the upper limbs. In resemblance to our patients, those three cases were reported to have generalized polyneuropathy which was most apparent in the legs. We did not observe any carpal tunnel syndrome in our patients.

With regard to the clinical pattern, and the onset and course of the disease, no differences were found between the hereditary cases and the so-called sporadic cases of primary amyloidosis with polyneuropathy. This is in agreement with reports about the histopathological findings (14). Our neurophysiological examination revealed quite similar findings in the hereditary and the sporadic cases.

From the neurophysiological point of view the findings were similar to those found in other long-standing polyneuropathies of different etiology (diabetes, uremia) and in the genetically determined "peroneal muscular atrophy" e.g. Charcot-Marie-Tooth's disease (7, 10).

Group II

Symptoms and/or clinical signs suggestive of polyneuropathy were present in seven of the nine cases in this group. In four cases the findings of the EMG examinations were interpreted as signs of polyneuropathy. The abnormalities were seen in the lower extremities and were most marked in the short toe extensor muscles.

The MCV was rather slow in the peroneal nerve on one or both sides in four cases. The velocities were borderline, not distinctly abnormal. In only one case (B:e) was the MCV of the peroneal nerve below 40 m/sec. The abnormalities of the EMG and MCV in that case were, however, restricted to the right leg and were presumably due to an earlier sciatic syndrome.

Clinically symptoms and/or signs of neuropathy were present in the upper extremities in four cases. The EMG of the first dorsal interosseal muscle and the MCV of the ulnar nerve were normal in all nine cases.

Thus in five cases the neurophysiological findings agreed well with the clinical evaluation. In two cases no clinical signs of peripheral polyneuropathy were found, and also EMG and MCV were normal. Three cases had both clinical and neurophysiological findings indicative of polyneuropathy.

In four cases the clinical symptoms and or signs suggested a neuropathy which was, however, not confirmed by the neurophysiological methods used in this investigation.

In no case did EMG or MCV reveal a neuropathy not discovered by careful clinical examination. It is, however, likely that more refined neurophysiological methods, such as single fiber EMG and estimation of sensory conduction velocities, could be valuable.

The diagnosis was not histologically confirmed in any of the nine cases. In members of a family in which amyloidosis is known to exist even minor neurological symptoms and or signs may of course be highly suggestive of early manifestations of the disease. Signs of denervation in EMG and or pathologically slow MCVs strongly favor the diagnosis. If, however, the neurophysiological findings are normal, we are apt to consider such

symptoms of polyneuropathy as inconclusive. Although peripheral polyneuropathy is a prominent feature in this form of amyloidosis, other symptoms and signs should be taken into account in the evaluation of each case, e.g. various visceral symptoms indicating disturbance of the autonomic nervous system gastrointestinal disorder with malabsorption, heart and kidney affection, and opacities of the corpus vitreum (3) In cases with clinical findings of peripheral polyneuropathy the manifestations were most apparent distally in the legs. In this group of cases EMG of the short toe extensor muscles was found to be a more useful examination than the estimation of MCV

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ADRENERGIC α AND β-RECEPTORS IN CORONARY VESSELS IN MAN

An In Vitro Study

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Abstract. The occurrence of adrenergic receptors has been investigated in vitro in isolated human coronary vessels from a newly dead 30-year-old man. Both noradrenaline and adrenaline easily contracted the originally atonic coronary vessels. This contraction was strengthened after blockade of the β-receptors with a specific adrenergic β-blocker (sotalol, MJ 1999). The contraction increased with the dosage and was completely blocked by an adrenergic α-blocker (dibenamine). Adrenaline had a somewhat stronger contracting effect than noradrenaline. The contraction was stronger in larger coronary vessels (diameter 2 mm) than in small (diameter 0.5 mm). On coronary vessels contracted by addition of potassium chloride to a final concentration of 13 μmoles/ml, both noradrenaline and adrenaline (1.7 × 10^{-6} g/ml) produced relaxation, the effect of adrenaline being somewhat stronger. After adrenergic β-blockade the relaxing effect of adrenaline and noradrenaline was completely blocked. The occurrence of both α- and β-receptors has thus been shown in human coronary vessels.

The effect of catecholamines on the coronary circulation in man is under discussion. The majority of the investigations in this field have been performed on animals, especially on dogs.

Most authors agree that catecholamines decrease the coronary resistance by stimulation of adrenergic β-receptors. A relaxing action on the coronary vessels may be produced by a stimulation of adrenergic β-receptors located in the smooth muscle cells of the coronary vessels and/or produced secondarily by a dilatation of the vessels as a consequence of stimulation of the receptors in the heart muscle cells, which leads to an increased myocardial metabolism caused by the positive inotropic and chronotropic actions of the catecholamines. Subsequent to blockade of adrenergic β-receptors in the heart a reduced coronary blood flow (CBF) has been observed in some species after administration of adrenaline or noradrenaline (4, 6, 9). It has been suggested that this vasoconstriction is caused by stimulation of adrenergic α-receptors in the coronary vessel wall.

In an earlier investigation (1) the effect of an adrenergic β-receptor blocking agent (sotalol, MJ 1999) on the coronary circulation and metabolism of the heart was studied under hypoxia and during infusion of catecholamines in the dog. Sotalol reduced the CBF and decreased the myocardial metabolism stimulated by the catecholamines and hypoxia. The adrenergic β-receptor blockade, however, increased the coronary resistance more than could be accounted for by a reduced myocardiac metabolism. Adrenergic β-receptor blockade increased the lactate/pyruvate quotient of coronary sinus blood in patients with angina pectoris and in volunteers during physical work (5, 11). This might be an indication of reduced aerobic metabolism of the myocardium, possibly dependent on an increased coronary resistance.

The question how catecholamines and adrenergic β-receptor blocking agents influence the tone of coronary vessels is therefore both of theoretical and practical importance and may be further elucidated by studying isolated coronary vessels.

Zuberbuhler and Bohr (14) and Bohr (2) have studied the effect of catecholamines on isolated coronary vessels from different species of animals

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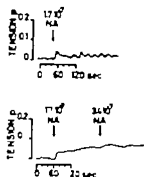


Fig. 1 *Upper* In vitro effect of noradrenaline (*NA*) on atonic human coronary vessel. *Lower* Effect on the same vessel after pretreatment with an adrenergic β-blocking agent, sotalol (MJ 1999· 1. × 10⁻ g/ml). The contracting effect is increased.

in itro. They demonstrated the presence of both adrenergic α- and β-receptors in the larger vessels. In small coronary vessels (up to 0.5 mm in diameter) adrenergic β-receptors dominated. After adrenergic β receptor blockade by pronethalol or propranolol the catecholamines did not regularly produce a contracting effect even in the large arteries. This might be due to the fact that the adrenergic blocking agents used by these authors, in addition to a β-receptor blocking effect, possess local anaesthetic and quinidine-like actions as well.

The occurrence of the adrenergic α- and β-receptors in isolated coronary vessels from man is unsettled As the receptors of the coronary vessels may exhibit species ariations (8) we have studied the occurrences of these receptors in isolated human coronary essels. A preliminary report of the present study and the study by Ekstrom Jodal et al. (5) was presented at the International Symposium on Myocardial Metabolism in Nancy France in 1969 (13)

METHODS

The experiments were performed on coronary vessels from patient, 33 years of age, ho arrived at hospital in moribund condition due to sleeping pills and lcohol intoxication. On arrival he was clinically dead, with respiratory and circulatory arrest. Superficial heart massage was immediately instituted but gav poor circulatory effect so that thoracotomy was performed. Heart massage was carried out for about 40 min without result. Thereafter the patient was declared dead by another physician and, 10 min later, in conjunction with suturing of the thoracotomy about 4 cm of the ventral branch of the left coronary vessel as well as a part of the surrounding tissue with branches from the larger vessel was excised. The preparation was immediately placed in chilled Krebs-Henseleit's bicarbonate buffer which had been aerated with 95% O_2 and 5% CO The large vessel with an inner diameter of about mm was divided into tw parts, each of which was clipped in a spiral manner and mounted in a special holder described by Lundholm and Mohme-Lundholm (7). In this holder the distance between the points of attachment of the preparation could be varied and the tension of the muscle layers was recorded by tension transducer (FT03) on Grass polygraph. The preparation and holder were placed in 30 ml Krebs-Henseleit's bicarbonate buffer at 37°C and aerated with 95% O_2 and 5% CO_2, the pH being about 7.4. About 30 min later when the preparation had been stabilized in the organ, both drugs were added.

RESULTS

The effect of noradrenaline on atonic coronary vessels before and after β-blockade

The coronary vessel had no basal tone. When noradrenaline was added, the vessel showed a short initial contraction (Fig. 1) which was followed by a relaxation and a more moderate increase of tone. After pretreatment with sotalol (MJ 1999) in a concentration of 1.2×10^{-8} g/ml this secondary relaxation was blocked and noradrenaline produced a persisting contraction.

The effect of noradrenaline adrenaline and adrenergic β-blocking agent on contracted coronary vessel

The atonic coronary vessel was contracted by the addition of potassium chloride to a final concentration of 1.3×10^{-2} M. Addition of nor adrenaline and adrenaline in a concentration of 1.7×10^{-7} g/ml induced in both instances a relaxation (Fig 2) Adrenaline relaxed the vessel almost completely while noradrenaline only reduced the tension by one half After adrenergic β-receptor blockade by sotalol the relaxing effect of noradrenaline was blocked and the tension in creased more than after potassium chloride

Adrenergic α-receptors in coronary vessels in man

The effects of noradrenaline and adrenaline on a large coronary vessel (diameter 2 mm) and on a small vessel after adrenergic β-receptor blockade are shown in Fig 3 In the large vessel nor

adrenaline produced a contraction which increased in relation to the dose. The effect was entirely blocked by an α-blocking agent, dibenamine (5×10^{-6} g/ml) Adrenaline also caused a contraction which was even stronger than that brought about by noradrenaline. In this instance the muscle too, relaxed completely after dibenamine.

The threshold concentration of noradrenaline with a contracting effect was higher in the smaller coronary vessel (Fig. 3) Again adrenaline was the most potent.

Effect of protriptyline

Protriptyline blocks the uptake of noradrenaline in the sympathetic nerve terminals. This is probably the most important inactivating route for noradrenaline (3) Protriptyline (5×10^{-6} g/ml) markedly potentiated the contracting effect of noradrenaline in vessels where the adrenergic β-receptors were blocked (Fig. 3).

DISCUSSION

The presence of both adrenergic α- and β-receptors in human coronary vessels is for the first time clearly demonstrated in this study

Treatment of patients with angina pectoris with adrenergic β-receptor blocking agent is usually accompanied by a reduction of the work of the heart and lowered cardiac oxygen consumption. This is a favourable action from a therapeutic point of view Blockade of the adrenergic β-receptors in the coronary vessels themselves, however obviously increases the constricting action of the adrenergic α-receptors. It

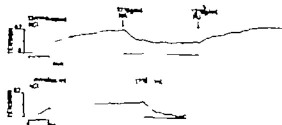


Fig. 2. Relaxing effect of noradrenaline (*NA*) and adrenaline (*A*) on human coronary vessel contracted by potassium chloride (1.3 10^{-4} M). Blockade of the relaxation after NA by sotalol (MJ 1999).

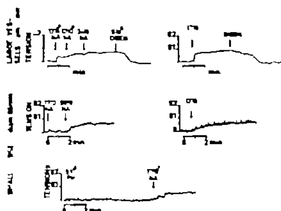


Fig. 3. Effect of noradrenaline (*NA*) and adrenaline (*A*) on human coronary muscle pretreated with sotalol. Upper curve: large vessel. Lower curves: smaller vessels. Diben = dibenamine, Ptr = protriptyline.

is probable that this effect is therapeutically unfavourable and may be deleterious, in some instances with fatal result (10 12)

Robin et al. (11) have found that in patients with angina pectoris propranolol increased the ratio lactate/pyruvate in the coronary venous blood, which may indicate a reduced oxygen supply to the heart muscle. Nitroglycerine had the opposite effect. In healthy subjects similar results have been obtained. During work on an ergometer bicycle adrenergic β-receptor blockade produced a lowering of the oxygen content of the coronary venous blood and increased the lactate/pyruvate quotient (5). It seems probable that after adrenergic β-receptor blockade the contracting effect produced by stimulation of adrenergic α-receptors in the coronary vessels is responsible for these metabolic effects.

From a theoretical point of view a combination of adrenergic α- and β-receptor blocking agents may have an advantage. A more generalized α-blockade combined with a β-receptor blockade will, however lead to a lowering of the blood pressure. An α-blocking agent with a specific action on the coronary vessels would probably be more favourable Nitroglycerine is often used in combination with adrenergic β-receptor blocking agent in the treatment of angina pectoris. By its direct relaxing effect on the smooth muscle of the coronary vessels it might prevent the constrictive effect elicited by adrenergic α-receptor stimulation

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THE EFFECT OF ADRENERGIC β-RECEPTOR BLOCKADE ON CORONARY CIRCULATION IN MAN DURING WORK

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Abstract. The effect of an adrenergic β-receptor blocker (sotalol) on coronary circulation and heart metabolism has been studied in six healthy subjects at rest and during work. Sotalol did not produce any uniform effects at rest. During work sotalol reduced the increase of cardiac output, oxygen consumption and coronary blood flow as well as the lowering of the total peripheral resistance which is produced by muscle activity. Despite the fact that sotalol reduced the work of the heart musculature and its oxygen consumption, oxygen extraction from coronary blood was increased. The pyruvate/lactate quotient in coronary venous blood was decreased during work by sotalol. The mechanism for reduction of metabolic vasodilatation by sotalol is discussed. It seems probable that after β-receptor blockade the contracting effect of adrenergic α-receptor stimulation gains an increased significance.

In earlier experiments with dogs (1) it was shown that an adrenergic β-receptor blockade with sotalol (MJ 1999) obviously inhibited the increase of cardiac work and oxygen consumption induced by catecholamines and hypoxia. The increase in coronary flow and the decrease of peripheral resistance in the coronary vascular bed which were connected with increased metabolism were also reduced. Certain facts indicated, however that sotalol also directly counteracted hypoxic vasodilatation. This direct effect of sotalol was most unmistakable in the vascular area of the femoral artery and confirmed earlier similar experiments by Folle and Aviado (8).

Considering the pharmaco-therapeutical significance of the adrenergic β-blocking drugs in the treatment of angina pectoris, it was of interest to investigate whether a similar effect of sotalol could be demonstrated in man. Therefore we have studied how sotalol influences coronary circulation and cardiac metabolism in healthy subjects, at rest and during exercise.

In these experiments we have placed special emphasis on attempting to reach an understanding of how sotalol influences the supply of oxygen to the cardiac musculature. Therefore we have determined the quotient pyruvate/lactate in coronary venous blood as well as oxygen content. This quotient is proportional to the quotient NAD/NADH and thereby gives information on the relation between oxidative and anaerobic processes in blood and tissue (10). A decrease of the pyruvate/lactate quotient indicates a decreased supply of oxygen in the tissues.

MATERIAL AND METHODS

The studies were performed on six healthy male volunteers, aged 23–28 years. The subjects fasted 12 hours prior to the study and received no premedication. After local anaesthesia polyethylene catheters were inserted percutaneously into a brachial artery and into a cubital vein. The vein catheter was introduced to the height of the clavicular region. Furthermore a cardiac catheter (Cournand 8) was advanced via an exposed cubital vein into the coronary sinus.

The coronary blood flow (CBF) was measured by the use of the inert gas diffusion technique of Kety and Schmidt (13, 14). A radioactive isotope of Xenon (Xe^{133}) was used as indicator. The arterio-venous difference of the indicator concentration was obtained during a saturation period of 13 min with simultaneous blood sampling from one brachial artery and the coronary sinus.

Simultaneous sampling was easier to obtain when the arterial catheter was connected with a cardiac catheter of suitable length giving the same volume as the venous catheter. This arrangement also had the advantage of minimizing the influence of a small affinity of the indicator to the catheter material (B. Ekström-Jodal et al.: unpublished observation).

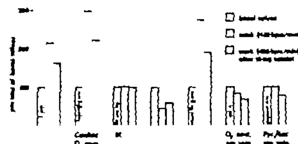


Fig 1 Percental changes in different parameters induced by a moderate work load before and after premedication with sotalol (10 mg i.v.)

The indicator was inhaled from a Tissot spirometer using a volume of 30–75 l Xe133-admixed air in a rebreathing system. The samples containing 2 ml of blood were then analysed for their γ-radiation in an automatic counter device (Gamma Automat, Kistner Sweden) The partition coefficient between cardiac tissue and blood was defined as 0.7 (6).

Cardiac output (C.O.) was measured by the dye dilution technique using the Beckman Cardiodensitometer for obtaining the dilution curve after injection of Cardio-green (Indocyanine-green). Separate calibration was performed for each subject

BPs were measured with strain-gauge manometers and recorded on a multi-channel Mingograf (Elema), and mean pressures were obtained by electrical integration. Three or more ECG leads were recorded continuously Oxygen saturation (12) and Hb concentration (7) were determined spectrophotometrically When calculating oxygen content, the Hb concentration of the arterial blood was used for both the arterial and the venous samples.

The micromethod of Siggaard-Andersen et al. (17) was used for determinations of pH and carbon dioxide tension. Lactate (15), pyruvate (5) and glucose (3) were determined enzymatically and free fatty acids (FFA) according to Trout et al. (20).

The studies were performed with the subjects in the supine position. After completion of the catheterization the subjects rested for 45 min. The first measurement of CBF was then made. Just before and after the flow determination C.O. was measured At the beginning and the end of the CBF measurement, simultaneous arterial and venous samples were taken for analyses of the oxygen content, pH, carbon dioxide tension and the concentrations of lactate, pyruvate, glucose and FFA. The heart rate was recorded continuously After this procedure three subjects were given 10 mg sotalol (MJ 1999) i.v. and the above mentioned measurements were repeated after 30 min.

In three of the subjects the initial measurements were repeated during exercise on an electrically braked bicycle ergometer (Elema) in the supine position at a constant work load of 400 kpm/min. After these measurements the subjects in this group rested for 15 min. The injection of sotalol was then given (10 mg i.v.) and work started again after another 10 min. The same measurements as before were repeated 10 min after the beginning of work.

RESULTS

As only three tests in each series have been performed, it has not been deemed necessary to make a statistical analysis of the material.

Basal conditions

The effect of sotalol on circulation and coronary metabolism during basal conditions was insignificant (Table I and Fig. 1). The changes were not similar in all three cases for any parameter A slight reduction of C.O. occurred in two of three cases and the pulse rate decreased in these cases. The total peripheral resistance (TPR) increased somewhat. The CBF was unchanged, as was the oxygen consumption of the heart. Lactate glucose and FFA uptake were not affected. These results agree with earlier studies in which it was shown that sotalol did not influence circulation in man under basal conditions (19).

Physical work

During a work load of 400 kpm for 30 min before sotalol C.O. increased in all cases after 5 min and the average increase was 7 l/min. The pulse rate increased by 31 beats/min Mean BP was not changed. TPR was reduced to about half of the initial value. CBF increased nearly three times and the resistance in the coronary vessels was reduced to one third. The oxygen content in coronary venous blood was slightly decreased in two of three cases and the oxygen consumption of the heart was tripled.

The uptake of lactate increased markedly as well as that of glucose and FFA. The pyruvate/lactate quotient in coronary venous blood remained unchanged.

When the subjects were premedicated with sotalol and performed the same work, the increase in C.O. in all three cases was lower than without sotalol. The increase in CBF was less after sotalol than before the decrease of peripheral resistance in the coronary vessels sank to 50% of the basal value. The increase of coronary oxygen consumption in all three cases was lower than before sotalol.

The uptake of lactate, glucose and FFA was increased in all cases in relation to basal values without sotalol.

Of particular interest were the changes in the pyruvate/lactate quotient and in oxygen content

Table I *Influence of sotalol (10 mg i.v.) on cardiac function and metabolism of the heart under basal conditions and under a constant work of 400 kpm/min*

Parameter	Rest Control	Rest Sotalol	Work Rest	Work Work	Work Work – sotalol
C.O. (l/min)	6.8	6.5	5.9	12.8	9.7*
Mean BP (mm Hg)	83	89	92	93	89
TPR (% of basal values)	100	111	100	47	60*
Pulse rate (beats/min)	65	62	69	100	94*
CBF (ml/100 g/min)	52	60	48	133	92*
Coronary resistance (% of basal values)	100	110	100	36	51*
O_2 content, coronary vein (Vol. %)	6.9	6.5	5.8	4.8	4.0*
O_2 extraction (Vol. %)	9.5	10.1	11.8	12.9	13.6*
Cardiac O_2 consumption (ml/100 g/min)	5.0	5.0	5.7	17.1	12.8*
Lactate arterial blood (mg %)	5.3	4.0	6.1	11.7	9.0*
Pyruvate/lactate quotient					
Arterial blood	0.12	0.11	0.11	0.08	0.09
Coronary vein	0.15	0.15	0.11	0.11	0.09*
Lactate uptake (mg/100 g/min)	—	—	0.5	6.5	3.0
Glucose uptake (mg/100 g/min)	—	—	7.0	14.5	8.3
FFA uptake (mg/100 g/min)	—	—	0.5	8.5	3.0

* Sotalol had the same action on the parameters in all three tests in the orking subjects.

in coronary venous blood. In arterial blood the quotient increased significantly during work after sotalol, despite that, the quotient in the coronary venous blood was lower during work after sotalol in all three cases. The oxygen content in coronary venous blood was lower during work after sotalol than before in all three cases, despite the fact that the heart work and oxygen consumption were reduced.

DISCUSSION

At basal conditions sotalol did not produce any uniform effects on the circulation or cardiac metabolism. Several interesting effects were found during work, however. The increase in C.O. was throughout lower at the same total physical work after sotalol than before and the decrease of the TPR was less. In view of the experimental conditions it was deemed necessary to complete the measurements within a reasonably short time. Therefore the second period of exercise had to be performed within 30 min after the first, in spite of the fact that repeated work is known to influence central hemodynamics within about this time period (4). The contribution of this influence to the present results is, however, considered to be rather small (9). A large part of the sotalol effect on the C.O. probably should be ascribed to a reduction of the stroke volume, as the pulse rate increase during work was inhibited only to a minor degree by sotalol.

The reduction of the increase of C.O. during work induced by sotalol was also combined with a less pronounced increase of the heart musculature oxygen consumption and coronary flow. Despite the fact that the heart oxygen consumption was reduced by sotalol, the oxygen content in coronary venous blood was lower after sotalol than before. As a further sign of the reduction of oxygen tension in the tissues, it was found that sotalol increased the pyruvate/lactate quotient in coronary enous blood, an effect which Robin et al. (16) have earlier shown to be produced by propranolol in angina pectoris patients at rest. This study indicates that, while sotalol reduces the work of the heart musculature and its oxygen consumption during physical work, it also reduces the metabolic asodilatation in the heart musculature and probably also in the skeletal musculature, since the lowering of TPR during work was decreased by sotalol. Sotalol therefore seems to have similar effects in man as ha e been earlier demonstrated in dogs (1).

An explanation for the reduction of the metabolic vasodilatation observed after sotalol adminis-

tration may be that, after blockade of adrenergic β-receptors in the vascular wall (stimulation of which produces a relaxing effect) the contracting effect of adrenergic α-receptor stimulation was magnified. This hypothesis has been tested in in vitro studies on human coronary vessels. It was found in vessels without tonus that adrenalin and noradrenalin had a weak contracting effect. If the vessel was contracted by potassium a strong relaxing effect was seen. After treatment with sotalol there was an obvious strengthening of the contracting effect of catecholamines on atonic vessels while the relaxing effect was blocked. The contracting effect was nullified by the α-receptor blocking agent dibenamin (2). This study therefore indicates that human coronary vessels possess both adrenergic α- and β-receptors.

Against the background of the above mentioned in vitro results the present findings can, in spite of the small material, be interpreted as most likely due to an α-adrenergic receptor stimulation unmasked by β-blockade.

ACKNOWLEDGEMENTS

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C-CELLS IN NON MALIGNANT HUMAN GOITERS STUDIED IN FINE NEEDLE ASPIRATION BIOPSY SPECIMENS

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Abstract. It was recently shown that in May-Grünwald-Giemsa stained smears obtained from thyroid medullary carcinomas by fine-needle aspiration the malignant C-cells have a very distinctive morphology with a bright red cytoplasmic granulation and rather characteristic nuclei as specific features. This enabled the author to detect corresponding cells in fine-needle aspirates also from non-neoplastic goiters. These presumed C-cells proved to be more common in toxic goiters than in cases of lymphoid thyroiditis and atoxic goiters without signs of malignancy or thyroiditis. In toxic goiters their occurrence showed a statistically significant positive correlation with the (^{131}I) T_3-uptake. They also were found to be more scarce in older than younger age groups.

The presence of two different cell types in the thyroid was first suggested in 1876 by Baber (4), who distinguished "the parenchymatous cells" with large nuclei, several nucleoli and fine granular cell substance from the bulk of epithelial cells in dog thyroid.

In 1932 Nonidez (17, 18) gave a more detailed description of a separate cell population in the dog thyroid characterized by argyrophilia and a parafollicular position. Interest in this second cell type of the thyroid has increased considerably in recent years and it has been shown that cells in a parafollicular or epifollicular position have many properties in common that distinguish them from follicular epithelium (23). These properties include monoaminergic mechanisms, ultrastructural characteristics and several characteristic histochemical reactions. Also the production of the calcium-lowering hormone thyrocalcitonin has been traced to these cells. Pearse (21) introduced the term C-cells (C for calcitonin) for the above mentioned cell system. He also pointed out similarities between C-cells and other cell systems in the body producing polypeptide hormone and possessing monoaminergic mechanisms, and coined the term APUD-cells (amine precursor uptake decarboxylating) for such cells (22). It has been generally accepted that medullary carcinoma of the thyroid originates at least partly through neoplastic transformation of the C-cells (29) but there are only few reports about these cells in non-malignant human thyroid glands (3, 5, 9, 25, 27).

The cytological picture of medullary carcinoma in fine-needle aspiration biopsy specimens of the thyroid has been described in a previous paper (15). We found some characteristic features of the malignant C-cells, especially a red granulation in smears treated with the routine staining technique of May–Grünwald–Giemsa (MGG). These findings in combination with histological descriptions of C-cells have made it possible to recognize these cells also in MGG-stained fine-needle aspirates from non-malignant goiters. The present paper concerns the identification of C-cells in such aspirates.

MATERIAL AND METHODS

The investigation was based on biopsy smears from toxic goiters, diffuse lymphoid thyroiditis, and atoxic benign goiters without signs of thyroiditis. The biopsy specimens in each of these groups were obtained from consecutive cases among goiters subjected to fine-needle biopsies in the Medical Department of the Hospital of Lund after exclusion of some specimens that were found to be too poor in cells for the present investigation.

The material of toxic goiters comprised 118 cases. The patients' ages ranged from 13 to 81 years (mean 45). The ratio men: women was 18:100. Protein-bound iodine (PBI) in serum was determined with a Technicon autoanalyser in 116 patients, triiodothyronine uptake (T_3-test) by Nonken's method (19) in 101 patients, and radioiodine uptake of the thyroid in 89 patients. The diagnosis of hyperthyroidism was based on clinical findings and the result of at least

Fig. 4. Group of C cells (in right upper part) and follicular epithelium from a toxic goiter. × 160.

Fig. 5. C-cells from a toxic goiter. Note the long slender cytoplasmic offshoot from one of the cells. × 400.

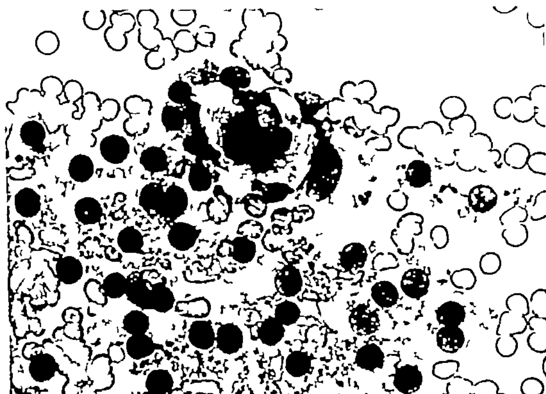


Fig. 6. C-cells among follicular epithelium from toxic goiter 256.

and nuclear appearance of some cells without distinct granulation seemed specific enough to permit their inclusion among C-cells by counting these cells. It was seldom difficult to distinguish C-cells from other types of cells. Doubtful cases were excluded from the C-cell count.

In HE-stained smears from goiters with rich occurrence of the above mentioned cell population, as judged from MGG-staining, some large cells were found with eosinophil granulation and elongated nuclei. The latter cells were probably identical with the presumed C-cells described in MGG-stained smears.

The frequency of presumed C-cells varied widely between the biopsy specimens. They were usually scarce and sometimes lacking altogether. However in some cases they constituted a few per cent of the cells counted. Table I shows that C-cells were more common in toxic goiters than in lymphoid thyroiditis ($p < 0.05$) and othe types of benign atoxic goiter ($p < 0.001$). In the series of toxic goiters C-cells were most common in younger patients and they were not found in specimens from patients above the age of 70. The mean age of hyperthyroid patients with demonstrated C-cells was significantly lower than of those without (Fig. 7). The same tendency of age distribution was seen among atoxic goiters with

Table I. *Occurrence of C-cells in three types of benign goiter*

χ^2 diff. biopsies with and without demonstrated C-cells between toxic goiters and lymphoid thyroiditis is 4.13 ($p < 0.05$) and between toxic and atoxic goiters without thyroiditis 13.3 ($p < 0.001$).

	C-cells demonstrated among 500 follicular epithelial cells		
Diagnosis	>5	1 5	0
Toxic goiters	31	15	52
Lymphoid thyroiditis	4	5	18
Atoxic benign goiters without signs of thyroiditis	2	2	26

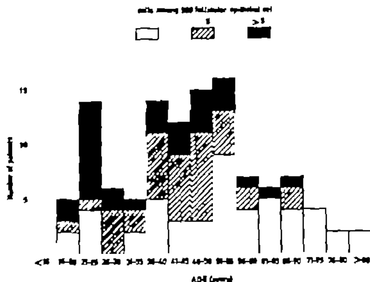


Fig 7 Age distribution by varying degrees of C-cell occurrence in toxic goiters. Mean age for patients with demonstrated C-cells is 40.0 ± 1.7 years, for patients without demonstrated C-cells 52.0 ± 2.5 years (*t* diff. 4.18 $p < 0.001$).

and without lymphoid thyroiditis. In toxic goiters C-cells tended to be more common in women than in men but the difference was not statistically significant ($p < 0.20$)

In cases of toxic goiter no statistically significant association was found between C-cell occurrence and LATS-activity PBI, 24 hour radioiodine uptake in the thyroid, or the quotient between 2 and 24 hour radioiodine uptake. Nor was there any association between the occurrence of C-cells and blood calcium levels. Goiters in patients with relapse of hyperthyroidism revealed no increase in the frequency of C-cells when compared with other toxic goiters. On the other hand, there was a statistically significant correlation between C-cell occurrence and T_3-uptake (*t* for difference between patients with and without demonstrated C-cells was 2.37 $p < 0.02$) The 2 hour radioiodine uptake was higher in patients with demonstrated C-cells than in those without (*t* for difference 3 10 $p < 0.01$) However the 2 hour radioiodine uptake revealed a linear correlation with age of the toxic goiter patients ($r = 0.43$ $p < 0.001$) and statistical covariance analysis showed no association between C-cell occurrence and the 2 hour radioiodine uptake independent of age.

DISCUSSION

The author s main argument for the identity between the cell population described here and C cells is its close similarity with the tumor cell population in medullary carcinoma as this appears in aspirate smears. Red cytoplasmic granulation in smears stained with the MGG technique, large often eccentric nuclei, and tenecncy to form bi- or multinucleated cells are common to both these cell populations. The cytological appearance of the presumed C-cells in smears is also in accordance with their appearance in histological sections, in which they have been described as large, often multinuclear cells with irregularly formed cytoplasm and large, often elongated, nuclei. In a previous investigation (6) an attempt was made to identify C-cells in biopsy smears from surgical goiter specimens perfused with *l* 3-4-dihydroxyphenylalanine (*l*-dopa) which induces a specific fluorescence in such cells. In spite of unsatisfactory staining with MGG after treatment with formaldehyde for the fluorescence study the identity between fluorescent C-cells and cells described in the present paper could be reasonably secured also by this approach.

Comparison of the occurrence of C-cells in different biopsies has some inherent sources of error First, the distribution of C-cells in the human thyroid is very uneven, most of the cells occurring in the central part of the gland (6). Second, the proportions between different cell types may be different in the smears than in the tissue itself because some cells are fixed more strongly in the tissue than are other cells and are thus more difficult to aspirate. It is possible that the higher vascularity of toxic than of atoxic goiters may influence the tendency of different cells to be aspirated and thereby their proportions

in the smears. However the observation of more C-cells in smears from toxic than from atoxic goiters fits in whith the results of *l*-dopa-perfusion studies of human goiters, in which the proportion of C-cells was largest in toxic goiters (6). An increase in the number of C-cells relative to the follicular epithelium has also been found in rabbits treated with thiourea (12) as well as in pigs treated with thiourea or thyrotropin (31).

It is known (7, 11, 22) that in certain animals, such as the mouse, C-cells can produce the monoamines 5-hydroxytryptamine (5-HT) and dopamine (DA) by decarboxylation of the corresponding amino acids, 3-4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP). The formation of DA by dopa-decarboxylation also in man has been proved (6, 9). Melander (14) has shown that these monoamines, when given in large doses, increase radioiodine release in thyroxine-blocked and hypophysectomized mice. It might therefore be speculated that the higher occurrence of C-cells in toxic than atoxic goiters demonstrated in this investigation may be related to the different rate of hormone release from the follicular epithelium.

Biological assays of thyrocalcitonin (2, 10) have shown lower concentrations in toxic than in atoxic thyroid gland tissue, though the difference did not reach statistical significance. The hormone storage in C-cells is strongly dependent on calcium concentration (8) and is lowered by hypercalcemia, which is often found in thyrotoxicosis. Thus the apparent disagreement between the content of C-cells in toxic and atoxic goiters reported in this paper and results of calcitonin assays in such goiters may be explained in terms of hormone storage.

The negative correlation between the number of C-cells in the examined goiters and the age of the patients can only be the subject of speculation. It may be related to increased nodularity of the goiters in higher ages. Thus Englund et al. (6) found that nodular parts of both toxic and atoxic goiters are very poor in C-cells. The age distribution of C-cells found in the present investigation may also have something to do with the high occurrence of osteoporosis in higher ages, as also with the increase of serum alkaline phophatase activity found with increasing age in a normal population (16).

ACKNOWLEDGEMENT

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SPECIFIC DETERMINATIONS OF PROTHROMBIN FOR EVALUATION OF LIVER FUNCTION

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Abstract. Prothrombin has been determined specifically with a two-stage assay based on intrinsic activation of prothrombin, and the values compared with other methods for determination of prothrombin and related factors. It was found that prothrombin values in healthy subjects were not influenced by age or sex. Prothrombin and Normotest values in acutely intoxicated alcoholics, without liver cirrhosis or hepatitis, did not show significant differences from those of normal controls. Prothrombin values in patients with cirrhosis and hepatitis were correlated to Normotest and prothrombin-proconvertin values. In patients with cirrhosis prothrombin values were correlated to serum albumin concentration, in patients with hepatitis to glutamic pyruvic transaminase (GPT) values, and thus appeared to indicate the degree of liver damage. Prothrombin values obtained with the present two-stage method are not significantly influenced by antithrombin levels.

In the routine work of the hospital laboratory determinations of prothrombin and related factors are used for the diagnosis of liver diseases and vitamin K deficiency as well as for the control of oral anticoagulant treatment. The methods employed for these determinations are basically modifications of the one-stage prothrombin time by Quick et al. (25), which is a screening test for all factors involved in the extrinsically activated coagulation process, i.e. factors II (prothrombin), VII, X, as well as I (fibrinogen) and V. In the prothrombin-proconvertin (P-P) method and in the Thrombotest and Normotest devised by Owren et al. (21, 22, 23), fibrinogen and factor V are added to the test system. Hence factors II, VII and X are registered by these methods.

Specific assays of prothrombin have been used for investigations of patients with liver diseases (14) but the testing procedure has been complicated. Moreover specific prothrombin assays have been considered less sensitive than tests which register several of the related factors (6, 11, 13, 24, 26).

A simple specific two-stage assay of prothrombin was developed in our laboratory (16). This report concerns the application of the method for investigations of patients with liver diseases and of alcoholic abusers. Prothrombin in normal individuals of different age was also investigated and the influence of antithrombin on the prothrombin determinations was evaluated. Results from investigations of prothrombin during infancy and in subjects on oral anticoagulant treatment have been reported elsewhere (17, 18).

MATERIAL AND METHODS

Assay Methods

Prothrombin was determined with 1) the specific two-stage prothrombin assay previously described (16), based on "intrinsic" activation of prothrombin in a mixture of partially purified coagulation factors, using a freeze-dried, ready-made reagent, 2) in some samples (n = 20) also with a one-stage prothrombin assay according to Koller et al. (12).

Prothrombin-proconvertin (P-P) was determined according to Owren and Aas (22).

Thrombotest (21) and *Normotest* (23) were performed according to the directions of the producer.

Factor V was determined according to Wolf (29).

Factor IX was assayed according to Nilsson et al. (15).

Antithrombin III activity was determined according to Hensen and Loeliger (10).

When capillary blood was used, the results were not corrected for hematocrit variations, which in the material presented were not considered to influence the results significantly.

Standards

Individual plasma and capillary blood samples from 20 apparently healthy persons, predominantly men, aged 18–

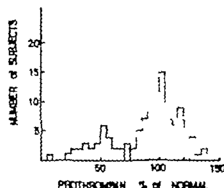


Fig. 1. Prothrombin in plasma samples from healthy subjects (–) and patients with hepatitis in the acute stage or with liver cirrhosis (---).

25 years, were used as standards for the two-stage prothrombin assay. The mean value of the determinations in these samples was designated 100% (16).

Mixed citrated plasma from 20 apparently healthy persons was used for the one-stage prothrombin assay and for the other coagulation factor assays.

Statistical Methods

Analysis of variance was computed according to B.M.D. programs 01V, 05V (Biomedical Computer Programs, University of California Press, 1965). Statistical analyses were carried out or supervised by the Department of Medical Physics of Karolinska Institutet.

Table I. *Prothrombin in normal apparently healthy persons*

Prothrombin values as thrombin U/ml incubation mixture under the condition of the test method

	Groups of normals			
	I	II	III	IV
No. of observations	20	20	18	18
$\bar{x}$	0.93	0.93	0.88	0.88
	0.13	0.12	0.13	0.15

Analysis of variance

Cause of variation	Sum of squares	d.f.	Mean square
Different sex	0.05109	1	0.05109
Different age	0.00015	1	0.00015
Interaction	0.00009	1	0.00009
Subtotal	0.05133	3	0.01711
Within groups	1.19589	72	0.01661
Total	1.24722	75	

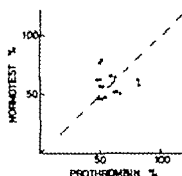


Fig. 2. Correlation of prothrombin and Normotest values in patients with hepatitis.

Clinical Material

The following groups of normal, apparently healthy persons, were investigated. 1) Plasma samples from (I) 20 men aged 18–22 years, (II) 20 women aged 18–25 years, (III) 18 men aged 67–80 years, and (IV) 18 women aged 67–80 years. 2) Capillary blood samples from 20 men aged 18–22 years and laboratory technicians, 9 men and 26 women, aged 22–44 years.

The following groups of patients were investigated: 1) Plasma and capillary blood samples from 15 patients, aged 19–70 years, with infectious or serum hepatitis from the Hospital for Contagious Diseases (Roslagstulls sjukhus) Stockholm. 2) Plasma samples from 11 patients with cirrhosis of the liver, aged 30–68 years, from Karolinska Sjukhuset and Serafimerlasarettet, Stockholm. 3) Capillary blood samples from 290 patients, aged 28–65 years, admitted to Mariakliniken, Stockholm (head: Dr R. Dunberg), for treatment of acute alcoholic intoxication. None of the patients had diagnosed liver cirrhosis or hepatitis. 4) Plasma samples from 13 patients, aged 8–62 years, with abnormal bleeding tendency and 1 patient with

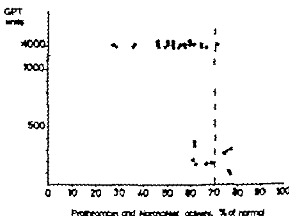


Fig. 3. Correlation of prothrombin and Normotest values to GPT activity in patients with hepatitis. GPT activity was determined according to Reitman-Frankel and expressed in Karmen-Wroblewski units. ○ prothrombin; ■ Normotest.

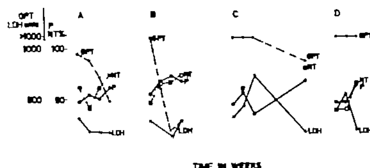


Fig. 4. Prothrombin, Normotest, GPT and LDH activity in 4 patients with hepatitis (*A*, *B*, *C*, *D*). LDH activity was determined according to Bobson-Phillips and expressed in IU/l. *P*: prothrombin, *NT*: Normotest.

thrombotic episodes, investigated at our laboratory. The patients with bleeding tendency showed prolonged thrombin time (2) but had varying antithrombin activity. The patient with thrombotic episodes had decreased antithrombin activity.

RESULTS

Prothrombin in apparently healthy persons

Prothrombin determinations (16) in plasma samples from 76 apparently healthy persons are summarized in Fig. 1. The mean value was 97.4% and the S.D. 13.7. The values showed approximately normal distribution, and calculation with logarithmic values gave similar results. Corresponding values for capillary blood samples from 55 normals were: mean 98.6% and S.D. 15.4% (Fig. 5). There was no significant difference ($p > 0.05$) in prothrombin values between the different age groups, nor between males and females (Table I).

Prothrombin in patients with liver disease

Prothrombin (16) and Normotest were determined in 15 patients with infectious or serum hepatitis. Plasma samples were used for prothrombin determinations, capillary blood for Normotest. Most of the patients were investigated several times. Prothrombin and Normotest values showed significant correlation ($r = 0.59$, $p < 0.01$) (Fig. 2). The prothrombin values were significantly inversely correlated ($r = -0.53$, $p < 0.05$) to the GPT values of the patients (Fig. 3). Serum albumin was not determined in these patients; thymol turbidity was not significantly correlated to prothrombin values. Results from four patients are shown in Fig. 4.

Moreover 11 patients with liver cirrhosis were investigated regarding prothrombin and P.P. and most of them also regarding factors V and IX, fibrinogen and platelet number (Table II). Fibrinogen was determined according to Blombäck and Blombäck (3), platelet count according to Kristenson (modif.), bilirubin by Nosslin's method (19). Serum electrophoresis was performed on paper at pH 8.6. The fractions were quantitated after elution with methanol. The thymol flocculation test was performed according to Maclagan. In all patients studied subnormal prothrombin values were found despite therapy with vitamin K. Prothrombin values were significantly correlated to serum albumin content ($r = 0.76$, $0.02 > p > 0.01$) but not to GPT values. There was no

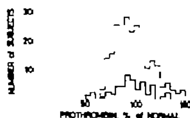


Fig. 5. Prothrombin in capillary blood samples from acutely intoxicated alcoholics ( ) and healthy subjects (—).



Fig. 6. Prothrombin ( ) and Normotest (—) in capillary blood samples from group of acutely intoxicated alcoholics.

Table II. *Prothrombin in relation to some other parameters in patients with liver cirrhosis*

Pat. no	Sex	Age (y)	Prothrombin (% of normal)	P-P (% of normal)	F IX (% of normal)	F V (% of normal)	Fibrinogen (g/100 ml)	Platelets/ µl	Bilirubin total (mg/100 ml)	Electrophor Alb (g/100 ml)	γ-glob. (g/100 ml)
1	♀	60	22	22	27	—	—	—	19.0	2.23	4 28
2	♂	59	29	26	50	30	0.15	92 000	6.6	(Thymol test normal)	
3	♀	43	31	36	—	—	—	57 000	3.5	3.49	2.98
4	♂	55	35	37	68	53	0.21	98 000	1.4	1.49	3.38
5	♂	40	40	40	—	37	0.27	62 000	11.2	(Thymol test normal)	
6	♀	35	40	41	55	42	0 16	—	1.5	2.81	2.66
7	♂	64	44	(TT = 39)	—	80	0 28	65 000	2.2	3.42	1.82
8	♂	68	53	49	52	98	0.30	80 000	1.0	3 69	3.70
9	♂	63	59	50	100	88	0 12	38 000	20.0	(Thymol test normal)	
10	♂	45	68	79	—	—	—	209 000	2.2	4.97	1.56
11	♂	37	69	59	82	98	0.42	35 000	1.5	2 9	1 7
			31	64	—	69	0.44	50 000	2.0		
Normal range			70–130	70–130	60–140	70–130	0.20–0.40	150 000–350 000	<1.0	4.0–5.4	0.7–1.5

P tient with liver cirrhosis and primary cancer of the liver Multitransfused patient.

appreciable difference between prothrombin and P P values. Factor IX was decreased in the pa tients with prothrombin ≤ 55% and factor V in the patients with prothrombin ≤ 40%. The platelet number was decreased in most of the patients and the fibrinogen concentration in some.

P othrombin in alcoholics

There was no significant difference ($p > 0.05$) be tween the mean prothrombin values for 290 utely alcohol-intoxicated patients and 55 nor al controls, nor between the distribution of values in the patient group and control group according to the Smirnov–Kolmogorov test (4) (Fig. 5)

Prothrombin and Normotest values (standardized against the same normals) were compared in 47 of the patients (Fig. 6) Mean value ±S.D was for Normotest 101±17% for prothrombin 102±18%. As can be seen, similar values were obtained with the two methods

Prothrombin in patients with different antithrombin levels

Prothrombin values obtained with the one- and two-stage methods (12, 16) were compared in 14 patients with varying antithrombin activity (10) (Table III)

In the patients with high antithrombin activity the mean prothrombin value obtained with the two-stage method was moderately lower than the corresponding value with the one-stage method. However the difference observed was not sta tistically significant ($p > 0.05$)

In the patients with normal or low antithrombin activity similar mean values were obtained with the two methods.

DISCUSSION

A two-stage assay of prothrombin based on in trinsic" activation of prothrombin (16) has been applied for investigation of patients with liver diseases. Compared to other specific assays, the method is simple and is rapidly performed with ready-made, standardized reagents. Capillary blood samples may be used. The assay is suited for estimation of high as well as low prothrombin levels.

Prothrombin values in healthy adults were approximately normally distributed and were not influenced by sex or age This is in accordance with the findings of other investigators using an immunological assay (8)

It has been advocated that a two-stage prothrombin assay should not be used for investigations of patients with liver diseases, as such assays are influenced by antithrombin (13) the activity of which shows extreme variations in these patients (6) Mannucci (13) found decreased anti-

Diagnosis confirmed by		Esoph. varic.	Shunt op.
Biopsy	Necropsy		
+	+	+	
		+	
+	+	+	+
		+	
		+	+
	+	+	
	+	+	
	+		
		+	
	+	+	

thrombin activity and higher prothrombin values with the two-stage method of Biggs and Mac farlane (2) than with a one-stage method in patients with liver cirrhosis. In patients with hepatitis Mannucci found predominantly increased antithrombin activity and lower prothrombin values with the two-stage than with the one-stage method. Contrary to these findings it could be shown that the influence of antithrombin on the present two-stage assay of prothrombin was negligible. The divergent results can probably be explained by methodological discrepancies. Undiluted plasma samples are used for the particular two-stage prothrombin assay (2) employed in Mannucci's investigation in contrast to our and to other two-stage methods (14 16, 28). In patients with established liver disease subnormal prothrombin values were found with the present method. In cirrhotic patients prothrombin values were significantly correlated to serum albumin concentration and hence to protein synthesis. In patients with hepatitis prothrombin values were correlated to GPT values.

The other coagulation disturbances found in the patients with liver cirrhosis, i.e. decrease of factors V and IX, fibrinogen and platelets, are in accordance with the reports of others (7 9 20). Whether these changes are caused mainly by deficient synthesis or by intravascular coagulation as well (1 6, 7) cannot be settled by the present investigation.

Table III. *Prothrombin in patients with varying antithrombin activity*

Pat. no.	Sex	Age (y.)	Diagnosis	Prothrombin Two-stage method (% of normal)	One-stage method (% of normal)	Antithrombin activity*
1	♂	42	Cardiac operation	71	68	High (140 %)
2	♂	50	Cardiac operation	60	72	
3	♀	54	Urinary bleeding	88	123	
4	♂	30	Spleen and kidney extirpation	52	81	
Mean				68	86	
5	♀	46	Mb Sternberg	45	34	Normal (64–136 %)
5	♀	46	Mb Sternberg	16	21	
6	♂	33	Malignant hypertension	127	127	
7	♂	33	Bleeding tendency	151	147	
8	♀	41	Willebrand' dis.	62	100	
9	♀	8	Urinary bleeding	151	127	
10	♀	45	Liver disease	49	56	
11	♀	44	Consumption syndrome	29	36	
Mean				79	81	
11	♀	44	Consumption syndrome	18	27	Low (< 60 %)
11	♀	44	Consumption syndrome	95	96	
12	♂	62	Ca ventr op.	46	44	
13	♀	59	Septicemia	43	47	
14	♀	23	Thrombophlebitis	151	151	
Mean				71	73	

Traces of heparin were not neutralized before testing the plasma samples.

A group of acutely intoxicated alcoholic abusers without diagnosed liver cirrhosis or hepatitis were investigated. Prothrombin was determined by the present method, and in some of the subjects Normotest was also performed. The prothrombin values of these patients did not differ significantly from those of the control group, nor did the Normotest values. Hence neither prothrombin nor Normotest values seem to be sensitive indicators of slightly impaired liver function which would be expected in some of these patients (27)

Assays sensitive to changes of factor VII, factor II and X, as well as of factor V have been recommended for evaluation of liver function (5 6, 7 11 24 26) From our investigation it appears that prothrombin determined by the present method, is a valuable indicator of the degree of liver damage and provides more specific information than tests sensitive to several factors.

ACKNOWLEDGEMENTS

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SPLEEN SIZE IN POLYCYTHEMIA

A Clinical and Scintigraphic Study

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Abstract. The size of the spleen has been determined in control subjects, in patients with polycythemia vera (PV) and in other forms of polycythemia. With ^{99m}T sulphur colloid and gamma camera technique the largest area of the spleen was determined and compared with the length of the spleen on X-ray examination and with results of palpation. The spleen scan area as shown to be enlarged in all but three cases of untreated PV (90%) and in all cases of PV in relapse. The group of absolute polycythemia other than PV had slight but statistically significant increase of the scan area compared with the controls. The group of pseudopolycythemia had scan area within the normal limits. In cases of active PV the size of the spleen was examined twice, at high hematocrit level and at normal hematocrit level, accomplished by repeated phlebotomies. The results did not show any statistically significant difference. There was, however, significant decrease in the size of the spleen after remission, induced by myelosuppressive therapy. Most normal-sized spleens were also judged as normal on X-ray examination, but within the normal range there was no correlation between the spleen scan area and the length of the spleen on X-ray. A fairly good correlation was found for enlarged spleens, but the residual standard deviation was rather large. Thirteen scintigraphically enlarged spleens had normal X-ray length. Twenty-three enlarged spleens and the normal-sized spleens could not be felt by palpation. A largest spleen scan area of above 81 cm^2 is regarded as above the upper limit of normal. A value above 104 cm^2 is in favour of PV when this disease is to be differentiated from secondary polycythemia.

The finding of an enlarged spleen is of great significance for the diagnosis of polycythemia vera (PV) and for the differentiation of this disease from other forms of polycythemia, especially when no leukocytosis or thrombocytosis is present.

An enlarged spleen is often easily detected by a well-done physical examination. However a slightly enlarged and sometimes even a grossly enlarged spleen may be beyond the examiner's perception (2) X-ray examination of the abdomen can often give a better evaluation of the size of the spleen but is of no help when the outlines of the organ are not clearly visualized (15) Radioisotope spleen scanning has been available now for some years as a diagnostic adjunct and Fischer (5) reported an extensive experience with spleen size determinations using ^{51}Cr-labelled heat-denatured erythrocytes and a traditional scanning procedure. The introduction of ^{99m}Tc sulphur colloid as a scanning agent (6) and gamma scintillation camera technique (14) ha e allowed an improved and more rapid visualization of the actual spleen size.

It has been suggested by several authors that in the early stage of PV the spleen might be slightly enlarged merely because of a passive distention of the organ due to an increase in the blood olume, not because of a reticulum cell proliferation (21 23) For the same reason some enlargement of the spleen might be expected in subjects with absolute polycythemia other than PV The degree of splenic enlargement which might be helpful for the differentiation of PV from controls and other polycythemias has thus not been defined.

The objects of the present investigation were: 1) To determine the size of the spleen with ^{99m}Tc sulphur colloid and a gamma camera technique in clear-cut cases of PV and other forms of

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Table I. *Age, largest spleen scan area and spleen length on X-ray in controls and in patients with PV and other forms of polycythemia*

	Age (y) (Mean ± S.D.)	Largest spleen scan area *n*	Mean ± S.D. (cm²)	Range (cm²)	Spleen length on X-ray *n*	Mean ± S.D. (cm)	Range (cm)
Controls	62 ± 17	22	57 ± 12	37–81	16	11.8 ± 1.6	10–13
Untreated PV	58 ± 16	28	136 ± 49	70–233	24	17.6 ± 3.5	12–24
PV in relapse	64 ± 7	11	135 ± 31	98–201	9	17.1 ± 1.6	15–19
PV in remission	63 ± 9	17	89 ± 22	42–121	8	13.8 ± 2.4	10–17.5
Other forms of absolute polycythemia	49 ± 15	9	76 ± 14	51–89	9	12.6 ± 1.8	10–15
Pseudopolycythemia	56 ± 15	12	63 ± 12	37–81	9	13.[illegible] ± 2.2	10–16

absolute polycythemia, and to compare the results with control subjects and a series of pseudopolycythemia. 2) To compare the results of the gamma camera technique with the X-ray findings and the results of physical examination. 3) To evaluate the effect of a decrease in blood volume on the size of the spleen. 4) To evaluate the effect of myelosuppressive therapy on the size of the spleen.

MATERIAL

Controls. This group consisted of 22 mainly hospitalized patients with minor cardio- and cerebrovascular disorders or uncomplicated peptic ulcer. They had no signs of cardiac failure, hepatic disease, infection, rheumatic disorder or any other condition involving the spleen. They were hematologically normal, and all had Hb values between 12.0 and 14.6 g/100 ml. The age distribution in this group was similar to the groups of PV patients (Table I).

Untreated PV. This group consisted of 28 cases, 16 men and 12 women, with a mean age of 58 ± 16 (S.D.) years. The diagnosis of PV was established by the presence of an increased red cell mass, measured with ^{51}Cr (at least above 36 ml/kg b.wt. for men and 32 ml/kg b.wt. for women), a normal oxygen saturation (≥ 92%) and a palpable spleen. If splenomegaly could not be detected on physical examination, at least two of the following criteria had to be present: 1) leukocytosis ≥ 12 000/mm³ in the absence of infection, 2) thrombocytosis ≥ 400 000/mm³ in the absence of bleeding and infection, 3) elevated leukocyte alkaline phosphatase activity ≥ 100 scores in the absence of infection, and 4) hypercellularity of the bone marrow (less than 10% fat) and megakaryocytic hyperplasia judged from a histological section. The mean red cell volume for men was 51 ± 10 (S.D.) and for women 45 ± 10 (S.D.) ml/kg b.wt. Fifteen patients had a palpable spleen on physical examination.

PV in relapse. This group consisted of 11 cases, 5 men and 6 women, with a mean age of 64 ± 7 (S.D.) years. They had been treated previously with myelosuppressive agents (busulphan or ^{32}P) and had either not responded to therapy or had been for some time in remission. At the time of examination all had active disease and fulfilled the diagnostic criteria of PV described above.

PV in remission. This group consisted of 17 cases, 7 men and 10 women, with a mean age of 63 ± 9 (S.D.) years. The diagnostic criteria for PV were the same as above. In all of them a reduction of the Hct to less than 50% had been induced by myelosuppressive therapy (busulphan, chlorambucil or ^{32}P) without the aid of phlebotomy. All patients had a normal peripheral white blood cell count and platelet count and no symptoms which might be ascribed to PV. In spite of continuous iron administration and absence of blood loss there was no rise in their Hct values.

Other forms of absolute polycythemia. The group comprised 9 patients, 8 men and 1 woman, with a mean age of 49 ± 15 (S.D.) years. The group included 2 cases with secondary polycythemia due to renal disease (hypernephroma, renal cyst), 2 with abnormal liver function tests but an unclassified hepatopathy and 1 case with abuse of alcohol but no obvious liver disease. In the remainder no obvious reason for the polycythemia could be observed in spite of careful investigation. All patients had an absolute increase of the red cell mass (m. 43 ml/kg b.wt., range 36–54 ml/kg b.wt.). The spleen was not palpable and the peripheral blood count was normal. Bone marrow histology was not characteristic for PV. Other tests, like serum uric acid, serum B_{12} and B_{12} binding capacity, leukocyte alkaline phosphatase activity were all normal. The arterial oxygen saturation was normal and there were no signs of cardiac failure.

Pseudopolycythemia. The group comprised 12 patients, all men. Their mean age was 56 ± 15 (S.D.) years. These cases had persistently elevated Hct values (≥ 52%), but their red cell mass was within normal limits. The mean red cell volume of this group was 32 ± 3 (S.D.), the mean plasma volume 38 ± 4 (S.D.) ml/kg b.wt. The peripheral blood count and bone marrow histology were normal. The spleen was not palpable. Leukocyte alkaline phosphatase activity and serum B_{12} were normal.

METHODS

Scintigraphy. The preparation of the ^{99m}Tc sulphur colloid was performed according to the method described by Larson and Nelp (9). The patients were not allowed to

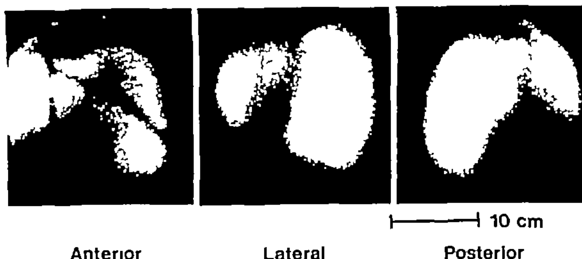


Fig. 1. Spleen scans from ^{99m}Tc scintigraphy in the three routine projections of case of PV with moderate splenomegaly. Lead strips were placed in the midline and along the left costal margin.

drink for two hours before the examination. Scanning was started about 30 min after the i. injection of 5 mCi of the colloid preparation, using gamma camera (Nuclear Chicago Pho-Gamma III), equipped with an 11-in. crystal and 4 000-hole collimator. A total of 500 000 counts was accumulated in every picture, for approximately 2 min. Three routine projections were made, left anterior upper quadrant, left posterior upper quadrant, and left lateral. In cases in which it was difficult to separate the liver and spleen areas from each other in the lateral scan, left anterior oblique (45°) projection was done. Fig. 1 shows the gamma camera image (scan) of the spleen in the three routine projections in case with moderate splenomegaly.

Completed scans were evaluated by measuring the spleen area in the lateral and posterior projections, where the highest activity over the spleen was normally found. The area of activity obtained on the photographic film (Polaroid) was sufficiently dense to allow an enveloping line to be drawn round its margin. The border of the spleen area was, however, not absolutely sharp, so that certain subjective error had to be expected. The standard error of single determination, calculated from duplicate measurements in 25 cases according to the formula $\sqrt{\Sigma d^2/2n}$ (4) was shown to be 3.2 cm which corresponds to coefficient of variation of 3%. All measurements were made by the same observer. With the help of an enlargement factor the measured spleen area in mm² was converted to cm². The largest spleen area from the two projections analysed was used as measure of the spleen size.

With the help of phantom studies, using spleen-shaped plastic reservoir filled with ^{99m}T solution, and absorbing material of different thickness, the basis for the calculations on the film was evaluated. The image that gave the most accurate measurements was one in which no parts were ove -exposed and the individual dots in the areas of maximum intensity could just be distinguished. The enveloping line had to be drawn just where the outlines of the image started to become blurred. Variations in spleen-collimator distance gave only slight differences in image size. This error could be minimized by using the lateral (or posterior) projection for the calculations, since in this position the distance is fairly constant from patient to patient.

Radiography. Plain films of the upper abdomen and of the left upper quadrant in the recumbent position were obtained with film-focus distance of 105 cm and without special preparation of the patient. Fluid intake was not llowed for two hours before the examination (1). The distance from the lowe tip of the spleen, long its length axis to the point where this axis reached the diaphragm was determined on the X-ray film. This length in cm was used as measure of spleen size. The readings were made by one and the same experienced radiologist, who had no knowledge of the clinical data.

Palpation. The palpability of the spleen was recorded in cm as the distance of the lower pole of the organ from the costal margin, 10 cm to the left of the midline. Palpation was carried out by at least two experienced observers.

Statistics. Standard statistical methods were used (19). Mean values were tested with Student *t*-test and the difference of means was considered to be significant if p 0.05.

RESULTS

Controls. The spleen size, measured as largest scan area, was in this group 57 ± 3 (S.E.) cm². Only one of the 22 cases had a scan area over 80 cm². The upper limit of the normal range was

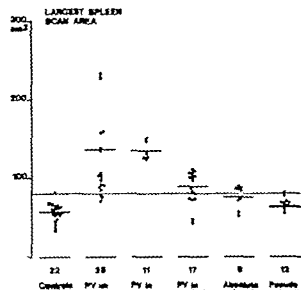


Fig. 2. The largest spleen scan area, determined with ^{99m}Tc scintigraphy in controls, PV and other forms of polycythemia. The horizontal bars denote the mean value in each group. The broken line marks the upper limit of the normal largest spleen scan area (81 cm²).

81 cm² (mean + 2 S.D.). There was no correlation between spleen size and body height, weight or surface area.

PV untreated and PV in relapse. The mean largest spleen scan area was similar in both these groups (136 ± 9 (S.E.) cm² and 135 ± 9 (S.E.) cm² respectively) and was significantly higher than in the controls and the other polycythemias. Fig. 2 shows that the values were distributed over a wide range in both groups of active PV. The largest spleen had a scan area of 233 cm². Only three cases of untreated PV had a scan area within the normal range. All cases of PV in relapse had a scan area above the normal range.

PV in remission. In this group the mean largest spleen scan area (89 ± 5 (S.E.) cm²) was significantly higher than in the control group but significantly lower than the means of both groups with active PV (Table I). The size of five spleens was within the normal range.

Eight patients were examined twice: before treatment and when in remission after treatment with ^{32}P (2 cases) or chlorambucil (6 cases). The results are shown in Fig. 3 B. A reduction in the size of the spleen was noted in all cases. The mean value *before treatment* was *132 ± 10* (S.E.) cm² and during remission 100 ± 4 (S.E.) cm². The difference between the means was statistically significant. Only one of these cases had a grossly enlarged spleen and this showed the largest reduction in size; for the remainder the reduction was moderate.

Other forms of absolute polycythemia. The mean value of this group (76 ± 5 (S.E.) cm²) was significantly higher than the mean of the controls. However, only five of the spleens were above the normal range (81 cm²) and none had a larger scan area than 89 cm².

Pseudopolycythemia. The mean value (63 ± 3 (S.E.) cm²) was not significantly different from the mean of the controls and all patients in this group had a spleen size within the normal range (Fig. 2).

Effect of phlebotomy on spleen size. From the group of untreated PV nine cases with a high Hct level and only slightly enlarged spleens were selected for study. Three had spleens within the upper normal range, the others were slightly to moderately enlarged. The size of the spleen was determined twice, at a high Hct level and after lowering the Hct to normal by repeated phlebotomies. The venous Hct at the first examination was 54–78%, mean 62%. Phlebotomies were performed in amounts varying from 2–5 l of blood

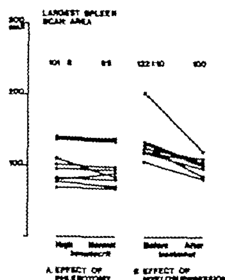


Fig. 3. (A) Effect of phlebotomy on the spleen size in nine patients with active PV examined at a high (≥ 55%) and normal (< 50%) Hct level. (B) Effect of myelosuppression on the spleen size in eight patients with active PV examined before treatment and during remission. The figures are means and standard errors of the largest spleen scan area for the respective group.

until the Hct was lowered at least to 50%. No myelosuppressive therapy was given. At the second examination the Hct varied between 42 and 49% mean 46%. The time interval between the two examinations varied between less than one month and five months.

Fig. 3 A shows the results of the spleen size determinations on both occasions. The mean value of the spleen scan area at the high Hct level was 101 ± 8 (S.E.) cm and after phlebotomy at a normal Hct level 95 ± 8 (S.E.) cm². The difference was not statistically significant.

Relation between lateral and posterior scan areas. Fig. 4 shows the correlation between the spleen scan area in the lateral and posterior projections in 97 examinations. In the region of normal-sized spleens the lateral and posterior scan areas were as a rule of the same magnitude. However in enlarged spleens the observations were almost invariably located to the right of the identity line, indicating that with enlargement the lateral area increases proportionally more than the posterior. In cases with extremely enlarged spleens the anterior projection of the organ was usually of the same size as the lateral. Only five cases with splenomegaly had a posterior scan area larger than the lateral.

Relation between spleen scan area and radiographic findings. Fig. 5 shows the correlation between spleen length in cm, measured on the X ray film, and the largest spleen scan area. For the controls the mean spleen length was 11.8 ± 1.6 (S.D.) cm (range 10–15). Fifteen cm was used as the upper limit (mean + 2 S.D.) of a normal-sized spleen. Most of the scintigraphically normal-sized spleens were also judged as normal on X ray. However within the normal limits there was no correlation between the largest spleen scan area and the X ray spleen length. Thirteen cases with enlarged spleens had an X-ray spleen length of 15 cm or less. Five of these spleens had a scan area abov 100 cm². The largest of them had a scan area of 159 cm and was also palpable 1 cm below the costal margin. In seven further cases the outlines of the spleen were not clearly visualized. These spleens were, however supposed by the radiologist to be normal-sized, and were also so on scanning except in one patient. For spleens with an X-ray length over 15 cm there was a fairly good correlation between the X ray length and the corresponding scan area (correlation coefficient = 0.68), but the residual standard deviation was rather large (35 cm²). Two normal-sized spleens were deemed as slightly enlarged by the radiologist.

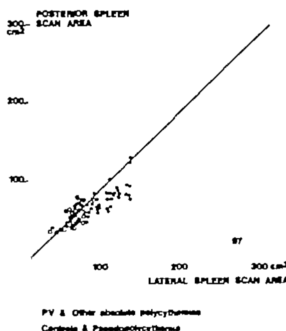


Fig. 4. Relation between the lateral and posterior spleen scan area, determined with ^{99m}T scintigraphy. Note that the large spleens are located to the right of the identity line.

Relation between spleen scan area and palpation findings. Fig. 6 shows the correlation between the results of the physical examination and the largest scan area. No normal-sized spleens could be felt by palpation, but 23 enlarged spleens could not be detected on physical examination. The largest of the latter had a scan area of 150 cm². The smallest palpable spleen had scan area of 97 cm. It may also be seen from Fig. 6 that there was a high degree of overlapping between the results of physical examination and those of scintigraphy.

DISCUSSION

The present investigation has shown that radioisotope scans can be used to obtain rapid and accurate information about the size of the spleen in every case where this information is essential for the diagnosis and for the follow-up of the patient. ^{99m}Tc as a scanning agent reduces the

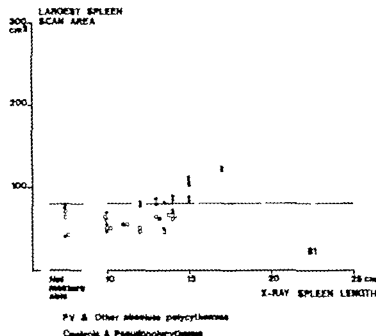


Fig. 5. Relation between the largest spleen scan area and the X-ray spleen length. For spleens with a length above 15 cm the equation of the linear regression was $y = 13.1x - 96.9$ $= 0.68$ $S_{yx} = 35.2$. For spleens with lower length no correlation was found between the two measurements. The broken line marks the upper limit of the largest spleen scan area for controls (81 cm²).

radiation dose to the patient to a minimum by its short half-life and pure gamma ray emission. The principal disadvantage of the present method, compared with the use of ^{51}Cr labelled heat denatured erythrocytes, is that the ^{99m}Tc colloid accumulates both in liver and spleen. This is not a drawback in the evaluation of normal-sized or moderately enlarged spleens, but in cases with huge spleens difficulty may arise in separating the areas of liver and spleen activity in the lateral projection. In these cases an anterior oblique projection can define more clearly the outlines of the spleen activity area.

The aim of our scintigraphic examinations was to obtain a measure of the spleen size which would enable us to separate enlarged spleens from spleens of normal size and to be able to follow the size of the organ in different phases of the disease in the same patient. The method is well suited for this purpose. With standardization of all involved technical steps, the main error of the method is confined to the definition of the line enveloping the area of activity in the gamma camera image. With the help of phantom studies and with a single observer making the measurements this error can be reduced to less than 5%.

Many authors (5, 17, 20) have tried to calculate the spleen volume or spleen weight from measurements of the spleen scan area in the lateral or

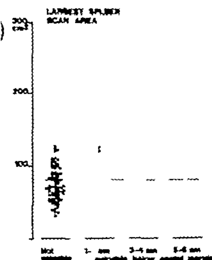


Fig. 6. Relation between the largest spleen scan area and spleen palpability. The broken line marks the upper limit of the largest spleen scan area for control subjects (81 cm²).

posterior projection, using ^{51}Cr-labelled heat denatured erythrocytes and a traditional scanning procedure. Different formulas have been proposed for this calculation. All of them make the assumption that the volume of the spleen can be expressed as a function of one cross-sectional area. Although these calculations, with the help of a proportionality factor have shown good correlation to spleen weight at operation and post mortem findings (8, 24) this procedure does not give more than an estimate based on a magnified error of the original determination of the spleen area. We therefore consider in accordance with recent article (18), that the estimation of spleen weight has little advantage for clinical purposes.

Splenomegaly is said to occur in PV in about 75% of cases (11 21). According to other authors the frequency is much higher (23) and according to Heilmeyer and Begemann (7) a normal-sized spleen in a case of PV must raise serious doubt as to the correctness of the diagnosis. Scanning has made it possible to define more exactly the size of the spleen and to determine the real frequency of normal-sized spleens in this disease. In the present PV series the measured spleen scan areas were distributed over a wide range. Most of the spleens showed a slight to moderate enlargement, none of them could be supposed to hav had a weight of more than 1 500 g. In the group of 28 patients with untreated PV three spleens had the largest scan area within the normal range. In the group of PV in relapse all spleens were enlarged. One common feature of the cases with active PV and a normal spleen size was the short known duration of disease. In the group of PV in relapse the smallest spleen scan area measured 98 cm² but all these cases had a known disease duration of more than 2 years.

It has been assumed that polycythemia antedates the enlargement of the spleen and that initially the spleen may be enlarged due to engorgement with blood because of an increased blood volume and not necessarily because of proliferation of the reticulum and its derivatives (21 23) The role played by an increase in red cell mass and total blood volume in producing splenomegaly in polycythemias of different etiology has not been studied sufficiently In the present study the largest spleen scan area was determined in nine cases of PV at a high Hct level and a second time when the blood volume and Hct level became normal after repeated phlebotomies. There was no significant difference between the means of the two measurements (Fig. 3 A) Only in one case was there a reduction in size, from 111 to 93 cm² in the other cases the changes were negligible. Five of the latter cases had spleens within the normal range or were only slightly enlarged. These cases were chosen for study since it could be supposed that their spleens were not affected by the myeloproliferative process to any great degree. The time interval between the two examinations varied between less than one month and five months.

In a group of nine cases of absolute polycythemia other than PV the mean spleen size was slightly but significantly larger than in the controls (Fig. 2, Table I) These findings might be interpreted as proof of a slight passive engorgement of the spleens. It should be observed, however that the number of cases is limited and that some of the cases in fact might be early cases of a myeloproliferative disorder not yet classifiable as PV according to our criteria. Roux et al. (16) have studied the spleen size in PV and hypoxic erythrocytosis and found very small overlapping between the two groups. However their series seems to include only one case of PV with a normal-sized spleen. The mean spleen weight for the cases with absolute polycythemia other than PV in their material was 195 ± 74 g. The mean spleen weight for the nine cases in our material, calculated according to the formula of Fischer (5) would be 193 ± 53 (S.D.) g.

Our results are in f our of the assumption that an increase in circulating red cell mass plays only a minor role in producing splenic enlargement in cases of PV and other forms of polycythemia, provided that portal hypertension is not present. It is most probable that the splenomegaly observed in PV is caused mainly by proliferation of the reticulum and extramedullary blood formation. The upper normal range for the largest spleen scan area in the controls was 81 cm² Spleens above this size may be regarded as enlarged. On the other hand we consider that the figure of 104 cm² which is the upper range of the spleen size for the group of absolute polycythemias other than PV might be more safely used as a limit for the differential diagnosis between PV and secondary polycythemia. The last figure is probably somewhat too high because, as

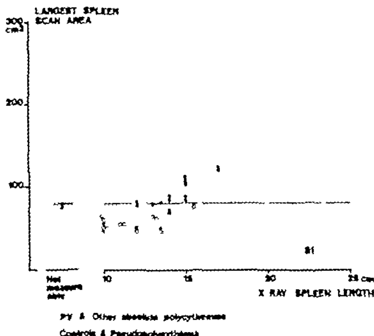


Fig 5 Relation between the largest spleen scan area and the X-ray spleen length. For spleens with a length abov 15 cm the equation of the linear regression was $y = 13.1x - 96.9$ $= 0.68$ $S_{yx} = 35.2$. For spleens with lower length no correlation was found between the two measurements. The broken line marks the upper limit of the largest spleen scan area for controls (81 cm²).

radiation dose to the patient to a minimum by its short half-life and pure gamma ray emission. The principal disadvantage of the present method, compared with the use of [51]Cr-labelled heat denatured erythrocytes, is that the [99m]Tc colloid accumulates both in liver and spleen. This is not a drawback in the evaluation of normal-sized or moderately enlarged spleens, but in cases with huge spleens difficulty may arise in separating the areas of liver and spleen activity in the lateral projection. In these cases an anterior oblique projection can define more clearly the outlines of the spleen activity area.

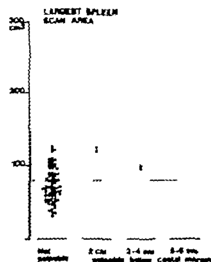


Fig 6. Relation between the largest spleen scan area and spleen palpability. The broken line marks the upper limit of the largest spleen scan area for control subjects (81 cm²).

The aim of our scintigraphic examinations was to obtain a measure of the spleen size, which would enable us to separate enlarged spleens from spleens of normal size and to be able to follow the size of the organ in different phases of the disease in the same patient. The method is well suited for this purpose. With standardization of all involved technical steps, the main error of the method is confined to the definition of the line enveloping the area of activity in the gamma camera image. With the help of phantom studies and with a single observer making the measurements this error can be reduced to less than 5%.

Many authors (5 17 20) have tried to calculate the spleen volume or spleen weight from measurements of the spleen scan area in the lateral or

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years. They usually lead to substantial impairment of vision in the long run, which apparently rather seldom results in complete blindness.

Various signs of vascular system involvement have also been observed in PXE. Thus, some authors have noted feeble or absent peripheral pulses, claudicatio intermittens, angina pectoris, and hypertension (5, 7, 14, 18, 22, 25, 34, 35, 41). Hemorrhages in various organs are also known to occur in PXE. Most common are the gastrointestinal bleedings, which may be very severe (15, 40). They have also been observed during pregnancy (30, 43). Subarachnoidal, retinal, renal and nasal bleedings (5) have likewise been reported.

From a pathologic point of view the typical PXE lesions, in the mid and the lower dermis, in the media of the vessels, and in Bruch's membrane are characterized by fragmented, thickened, and degenerated elastic fibers (16, 17, 20, 29, 31). Deposition of calcium in the elastic fibers is common and is regarded as an early change closely related to the unknown metabolic defect in PXE (?1).

More or less specific changes of the gastric mucosa have also been described. Thus, in certain cases the mucosa has been reported to be edematous, friable, ulcerative or even necrotic (7, 24, 25, 43). Microaneurysms (2), as well as fatty necrosis and calcification of the gastric vessels (40) have also been observed in cases of PXE complicated by gastrointestinal bleeding.

A number of studies on PXE have been published by Swedish authors during the last 20 or 30 years (5, 6, 7, 23, 38). Most of these papers are based on cases originally collected by Grönblad and carefully studied by her particularly from the ophthalmologic point of view.

With Grönblad's permission the present investigation is also based on her material. Our aim has been to study PXE mainly from the viewpoint of internal medicine, with particular reference to prognosis and late manifestations.

MATERIAL AND METHODS

The material on which the present study is based consists of 27 cases with PXE. Twelve patients, four male and eight female, were dead when our study was finished on Feb. 28, 1971. Fifteen patients, one male and 14 female, are still alive. One of the latter patients was described in Grönblad's dissertation in 1932 (23). Nineteen additional cases, also included in our study, were reported by Carlborg et al. (7). The remaining seven cases have not been published before.

All available written information about the various patients, such as hospital records, earlier descriptions (5, 7, 23), death certificates, autopsy records, have been collected and scrutinized by the authors. All the living patients, except one female, were examined by us at Medical Department III of St. Erik Hospital during 1969–71.

In addition to having their detailed histories recorded and undergoing a physical examination, all these patients were subjected to ECG and X-ray of the heart, lungs, skull, and extremities, as well as to the following laboratory tests according to conventional analytical methods: estimation of serum Hb concentrations, vitamin B_{12}, folic acid, creatinine, sodium, potassium, chloride, calcium, phosphor, magnesium, PBI, cholesterol, triglycerides, total lipids, SGOT, SGPT, LDH, bilirubin, alkaline phosphatase, blood sugar and uric acid.

The sera from the various patients were also investigated for the presence of antibodies to parietal cells, thyroid, kidney and for the presence of antinuclear factor.

Paper electrophoresis of serum, the T_3-resin test, qualitative tests for urinary protein and glucose, and microscopy of urinary sediments were also performed. In eight of the patients the 24-hour excretion of total hydroxyproline was determined according to the method of Bergman and Loxley as modified by Koevoet (27).

All the females, except one, were examined gynecologically. Finally, ophthalmoscopy was performed and visual acuity controlled in all the patients.

CASE REPORTS

In order to illustrate the natural history of the disease the following two, typical reports are given. Both patients, one with and the other without bleeding complications, were observed for a considerable length of time by us and by earlier Swedish investigators.

Case 22

(Case 2 in Carlborg et al. (7).) Female born in 1919. A sister has PXE. Typical skin lesions were detected when she was 7 years old. Diagnosis verified by biopsy in 1939. Examination in 1969 showed skin lesions on the neck, in the axillary folds and groins, the antecubital and popliteal spaces, and on the wrists and inframammary regions.

Angioid streaks were noted at the age of 30. The patient's vision was normal until the age of 48, when partial loss of vision occurred in the right eye. In 1969 visual acuity of the right eye was 1/60 and of the left 1.0. In the eyegrounds angioid streaks and small atrophies were found around the papillae.

Both in 1937, during a pregnancy which ended in spontaneous abortion, and again in 1945 hematemesis and melena occurred. X-ray of the ventricle and esophagus was negative. Blood transfusions were required. In 1946 the patient had melena. A gastric resection was made, but there was another period of melena in the same year. In 1969 a period of melena recurred, with discomfort in the epigastrium. X-ray of the ventricle, esophagus and colon was normal. No blood transfusion was needed.

Angina pectoris appeared at the age of 39. Claudicatio



Fig. 1 Broad vessel-like angioid streaks. Choroidal sclerosis (upper). Typical skin lesions (lower).

intermittens and muscular cramp in the calves have been present for several years. In 1968 a digitogram of dig. II pedis showed pathologic values with long propagation and inclination time and very low amplitude as an expression of very grave obstructive changes bilaterally. Oscillometry showed low amplitudes of the instep. At the clinical examination in 1969 the pulses of the radial arteries were diminished. The femoralis pulses were readily palpable, but distally no pulses were perceptible.

Physical examination of the heart was normal. BP was also normal. ECG showed signs of coronary insufficiency whereas X-ray of the heart, lungs and skull was normal.

Calcifications were observed in the arterial media in the arms and in the bottom of the lower extremities.

Laboratory examinations were normal, except cholesterol, triglycerides and total lipids, which were abnormally increased.

Case 13

(Case 3 in Grönblad's dissertation (23).) Male, born in 1904. One of the patient's sisters had skin lesions similar to his but no lesions in the eyegrounds. Skin lesions were noted in 1929 when angioid streaks were found, but the latter may have appeared earlier. Examination in 1969 showed skin lesions on the neck, in the axillary folds and groins. Diagnosis verified by biopsy.

In 1921 the patient's right eye was injured, causing rupture of the choroidea and retinal hemorrhage and resulting in partial loss of vision. In 1929 vision of the left eye was diminished. Angioid streaks were then observed and the diagnosis of PXE was established. During the following years there was progressive impairment of vision, and in 1969 visual acuity was 1/60 bilaterally. Angioid streaks,

Table I. *Causes of death and age at death*

Case no.	Sex	Age at death (y.)	Cause of death	Autopsy
1	♂	60	Apoplexia cerebri + Diabetes mellitus	–
2	♂	61	Infarctus cordis	+
3	♂	65	Haemorrhagia gastrointestinalis ex ulcerum mucosae gastrici	+
4	♂	75	Thrombosis cerebri + Ca pulm dx	+
5	♀	56	Myeloma	–
6	♀	59	Haemorrhagia cerebri	+
7	♀	61	Glioblastoma multiforme	+
8	♀	72	Hypertonia ess. benign. c. morbo cordis	–
9	♀	73	Encephalomalacia subchron. et acuta + Embolia pulm.	+
10	♀	82	Thrombosis cerebri recid + Cardiosclerosis + Diabetes mellitus	–
11	♀	84	Art. sclerosis myocardii + St. post haemorrhagia cerebri	–
12	♀	89	Carcinom. nephrosclerosis gravis + Hypertonia levis	+

Table II. *Skin and mucous membrane lesions of the living patients*

Mean age at manifestation (n = 8) 15 ± 3 y
+ = slight, + + = moderate, + + + = pronounced

Case no	Age at examination (y.)	Sex	Age at onset (y.)	Degree of skin lesions	Regions of affected mucous membranes
13	65		25*	+ +	—
14	78	♂	Childhood	+ + +	—
15	75	♀	16	+ + +	Portio
16	74		23	+	—
17	63	♀	15	+ +	—
18	59	♀	20–25	+ + +	Bucca
19	59	♀	10	+ + +	—
20	59	♀	45*	+ + +	—
21	57	♀	4	+ +	—
22	50	♀	7	+ + +	—
23	50		49*	+	Bucca
24	47		25	+ + +	Vagina
25	47	♀	18–20	+ + +	Vagina
26	36	♀	17	+ + +	—

* Skin lesions may have appeared earlier but were not observed.

and pronounced peripapillary and macular atrophy were present in the eyegrounds.

In 1967 hypertension was noted, and hemiparesis developed in the same year. Recovery from the latter has been good. He had no claudicatio intermittens or angina pectoris. The pulses of the femoral arteries and the dorsal artery of the right foot were readily palpable, but the pulse of the dorsal artery of the left foot was not palpable. The pulses of the radial arteries were feeble. Physical examination of the heart was normal. BP was normal after thiazide therapy. X-ray of heart and lungs showed moderate enlargement of the heart (510 ml m²). The lungs and the X-ray of the skull were normal. ECG showed slight signs of coronary insufficiency.

Slight medial calcifications were found in the vessels of the extremities. Laboratory examinations did not reveal any abnormalities with the exception of hypercholesterolemia and increased total lipids.

RESULTS

The various causes of death of the 12 deceased patients with PXE are indicated in Table I. Two patients died of malignant diseases, nine of cardiocerebrovascular diseases and one of gastrointestinal hemorrhage. The average age at death for the total material was 70 ± 3 years, for the four males 65 ± 5 and for the eight females 72 ± 4 years.

Two of the deceased patients were known to have suffered from diabetes mellitus (cases 1 and 10) and in one case the diagnosis of hyperthyroidism was established at the age of 72 (case 9).

Table III. *Latest known visual acuity of the deceased patients and age at onset of diminished vision*

Case no.	Sex	Visual acuity Right	Left	Age at onset (y)
1	♂	3/60	3/60	40
2	♂	2/60	1/60	49*
3	♂	1 1/2/60	1/2/60	48
4	♂	3/60	1/60	43
5	♀	4–5/60	4–5/60	38
6	♀	1/60	1/60	41
7	♀	0.1	Hand movements	31
8	♀	1/4/60	1/4/60	44*
9	♀	1/60	1/60	48
10	♀	1/2/60	1/60	49
11	♀	Not known	Not known	56*
12	♀	2/60	–/60	40

Signs that diminished vision may have appeared earlier

The average age of the 14 living patients examined was 59 years at the time of our investigation in 1969–71. Eleven of these patients are known to have close relatives with PXE: in nine cases siblings, in one the mother, and in one the maternal grandmother.

In eight patients PXE was first diagnosed when visual impairment occurred, in one patient at onset of first gastrointestinal hemorrhage, in another because of the skin lesions, in one in connection with the appearance of a gangrenous ulcer, and in yet another owing to establishing the diagnosis of PXE in a sibling. Two patients did not remember the circumstances which led to the proper diagnosis.

Skin lesions

In 11 out of 17 deceased patients skin lesions of typical appearance and distribution were reported. The age at onset of the skin lesions is not known in five cases. In the remaining patients the lesions were first observed between childhood and the age of about 30.

All the living patients had skin lesions. The age at onset varied from early childhood to 25 years of age in 12 patients, and in three patients it was not established (Table II). Mean age at onset was 15 years. All the lesions were located in typical areas and were regarded as slight in two patients, moderate in two, and severe in ten. Skin biopsies performed in 13 cases revealed the typical histologic PXE symptoms.

Typical PXE changes on the buccal mucosa were found in two patients, on the vaginal walls in two, and on the portio uteri in one (Table II).

Visual disturbances

All the dead patients except one, whose visual acuity is not known, had severe impairment of vision as listed in Table III, which shows the latest data obtained on the vision of the various patients. The impairment was first observed between the ages of 31 and 49 years. Angioid streaks are known to have occurred in all the patients.

In the living patients the onset of impaired vision came between 25 and 67 with mean age 43 years (Table IV). Only three of the patients had normal vision at the time of our study, whereas in eight cases a very marked decrease of visual acuity was noted. In most patients there was apparently a slow progressive impairment of vision and at the age of 70 the visual acuity of all patients was 0.2 or less.

Angioid streaks were present in all the patients. In those with impaired vision different degrees of macular, peripapillary atrophy and of retinal-pigment proliferation were also observed.

Table IV. *Visual disturbances in living patients*

Mean age at manifestation (n = 11) 43±5 y

Case no.	Age at examination (y)	Sex	Age at onset (y)	Visual acuity at examination Right	Left
13	65	♂	25	1/60	1/60
14	78	♀	43	0	0
15	75	♀	67	1/60	0.3–0.4
16	74	♀	47	3/60	1/60
17	63	♀	45	0.1	2/60
18	59	♀	43	Hand movements	Perception
19	59	♀	25	Hand movements	Hand movements
20	59	♀	39	1/2/60	0.1
21	57	♀	44	0.7	0.9
22	50	♀	48	1/60	1.0
23	50	♀	—	1.0	1.0
24	47	♀	45	Hand movements	Hand movements
25	47	♀	—	1.0	1.0
26	36	♀	—	1.0	1.0

Table V *Signs and symptoms of cardiocerebrovascular disease*

		Case nos.	Mean age (y)	Mean age at onset (y)	Remarks
Angina pectoris	5	14, 17, 21, 22, 24	59±6	39	
Hypertension	2	13, 16	69	—	220/95 200/110
Stroke	1	13	63	63	
Pathologic ECG (at rest) indicating coronary insufficiency	7	13, 14, 16, 17, 19, 21, 22	63±4	—	Atrial fibrillation in case 20. AV block I in case 15
Enlargement of the heart	5	13, 14, 16, 18, 21	66±4	—	♂ > 500 ml/m² ♀ > 450 ml/m²

Cardiovascular diseases

Among both the deceased and the living patients (Table I) a high frequency of signs and symptoms of cardiovascular disease was noted (Table V). Thus, five of the patients examined, all females, had a history of angina pectoris early in life, at a mean age of 39 years at onset. One patient (case 24) seemed to have suffered from angina pectoris from the age of 18. Four of the patients with a positive history of angina pectoris also had ECGs which were consistently interpreted as indicating coronary insufficiency. On the other hand no patient seems to have suffered from cardiac infarction. Slight to moderate enlargement of the heart, of unknown etiology was observed in five patients, hypertension in two, one male and one female. The former had also experienced an episode of hemiparesis with good restitution. Atrial fibrillation was present in one case.

Table VI summarizes the signs and symptoms of peripheral vascular disease in the PXE-patients. Thus, claudicatio intermittens was found to have occurred at an early age in four patients, whose mean age at onset was 32. The youngest patient at onset was a girl (case 24) who had suffered from typical intermittent claudication from the age of 18. At the age of 32 a gangrenous ulcer developed on her right foot. When examined at the age of 47 her left femoral pulse was readily palpable, whereas the right was feeble, and the pulses below this level were absent. X-ray showed moderate intimal and slight medial calcifications of the arteries of the lower extremities.

Eight patients complained of muscular cramp in the calves. Absence or peripheral arterial pulses on either one or both sides was also noted in several cases, as well as the presence of vascular calcifications demonstrated by X-rays of both the upper and lower extremities (Table VI). X ray examination showed the absence of calcifications in both the intima and the media only in three patients.

In two patients (cases 15 and 20) calcifications of the internal carotid arteries were also visualized by X rays of the skull. These patients, and one other (case 26) also had calcified falx cerebri.

Gastrointestinal bleedings

Among the deceased patients one male (case 4) had gastrointestinal bleeding from a gastric ulcer which proved fatal. No microscopic examination was made.

Among the living patients, three females have had gastrointestinal bleedings. Thus, in one patient (case 22) five episodes of severe gastrointestinal hemorrhage occurred, three with melena and two with both melena and hematemesis. In this patient the first bleeding took place when she was 17 and the last when she was 50. One gastrointestinal hemorrhage occurred during an early pregnancy which ended in spontaneous abortion another, her third, was followed by a resection of the stomach according to Billroth II which, however, did not prevent further episodes of bleeding. In this patient X-ray studies of the gastrointestinal tract failed to reveal the source of bleeding of any of the episodes.

The second patient (case 18) had her first period of gastrointestinal bleeding, characterized by both melena and hematemesis, at the age of 20.

Table VI. *Signs and symptoms of impaired peripheral circulation*

		Case nos.	Mean age (y)
Intermittent claudicatio	4	17 21 24 25	54±4
Muscular cramp in the calves	8	14 17 18, 19 20, 21, 22, 23	59±3
Absent or feeble pulses			
A. radialis	10	13, 14, 18, 19 21, 22, 23, 24 25, 26	55±4
A. femoralis	8	14, 15 17 18, 21 22, 24, 25	60±4
A. dors. pedis	10	13, 14 15 17 18, 21 22, 24, 25, 26	58±4
A. carotis	4	14, 21 24, 25	57±7
Calcifications of vessels (X-ray)			
Upper extremities			
Media	8	13, 15, 16, 18, 19 21 22, 24	61±3
Intima	1	18	59
Lower extremities			
Media	9	13 14, 15, 16, 17 18 19, 21 24	64±3
Intima	9	14, 15, 17 18, 19 21, 22, 24 25	59±4
Carotis	2	15, 20	67

X ray examination of the gastrointestinal tract was normal. There was a relapse, with severe hematemesis and melena, when she was 47 The only positive X-ray finding was then deformation of the duodenal bulb. In the third patient (not examined by us) the first, and so far the only bleeding occurred when she was 55 Both hematemesis and melena were present. Laparotomy [illegible]ved that the bleeding spot was on the minor curvature 3 cm below the cardia, and was described by the surgeon and the pathologist as follows. Macroscopically: An artery diameter 1 mm, was found to contain a clot which, when removed, caused bleeding. A section of the gastric wall, 4–5 × 3 cm, was excised. Several wide, pathologic arteries were seen in the submucosa the widest had a diameter of 3 mm. Microscopically: A large, tortuous artery with a very fibrous and thickened intima, was observed. It did not engage the muscularis propria. Calcifications were noted in both the intima and the media. In some parts of the media there was also fibroelastic proliferation. The elastic substance was partly destroyed in the lamina elastica interna, but was preserved in the lamina elastica externa. The veins showed no changes in the elastic substance. In the submucosa an artery of muscular type was found with a thick, probably somewhat hyperplastic, lamina elastica interna and externa, without any degenerative changes. No atherosclerosis was found.

Endocrinopathy

Four patients (cases 17 20 24 and 25) all females, were found to have atoxic goiter Hyperthyroidism, treated with antithyroid drugs, had occurred in one patient (case 18) On the other hand none of the living patients in the PXE material had had diabetes mellitus or any other form of endocrinopathy

Renal symptoms

The renal function was unaffected in all the living patients with PXE, except for one (case 14) who had a slight impairment. She suffered from bilateral nephrolithiasis. Another patient (case 23) had had ureterolithiasis and macroscopic hematuria on two occasions.

Neurologic disturbances

Two of the patients had experienced periods of vertigo. In one (case 25) ENG indicated neuritis of the vestibular nerve, and in the other (case 16) a spontaneous nystagmus to the right side was detected. A third patient (case 17) presented signs of parkinsonism, possibly secondary to cerebrovascular involvement.

Psychiatric disorders

Debility (IQ 67 Terman-Merrill 1948) was observed in one patient with PXE (case 21) Two

other patients had been treated in psychiatric clinics for mental depression (cases 16 and 24).

Laboratory findings

The extensive laboratory screening of the PXE material, with regard to a series of chemical and immunologic tests, showed very few abnormalities. In four patients (cases 13, 18, 21 and 22) hypercholesterolemia (> 300 mg%) was detected. Two of these patients (cases 21 and 22) and two further patients (cases 14 and 24) also showed increased triglyceride serum levels (> 2 mmol/l). Three patients (cases 13, 21 and 22) had also total lipids exceeding 0.9 g%.

Antibodies to thyroglobulin of a fairly low titer were found in one patient (case 20) who also showed a positive reaction to antinuclear factor in serum. No signs or symptoms indicating thyroid disease were observed in this case.

In two other patients (cases 15 and 24) low titer antibodies to parietal cells were present. These patients, however, had no history of gastric disorders.

Table VII shows the results of the determination of hydroxyproline excretion in eight patients with PXE. Two of them had increased hydroxyproline excretion, and in six patients total hydroxyproline excretion was within normal levels.

Table VII. *Urinary excretion of hydroxyproline*

Case no.	Age (y.)	Sex	Hydroxyproline (mg/24 h.)
15	75	♀	42.1
17	63	♀	17.0
19	59	♀	23.1
21	57	♀	48.0
			47.0
22	50	♀	14.3
4	47	♀	18.3
25	47	♀	24.3
			27.3
Normal range			14.9–33.0

DISCUSSION

Contrary to many other studies on PXE reported in the literature, all the patients dealt with in this paper showed typical dermal and ocular lesions. Consequently, they all fulfilled the criteria of the Grönblad–Strandberg syndrome.

The material under review includes most of the cases of PXE reported in Sweden. Moreover, most of the patients have been subjected to clinical observation by various investigators for a considerable length of time. Thus, the diagnosis of PXE was established between 4 and 32 years before death in 12 patients, and for the 15 living patients the observation period varied between 6 months and 40 years. For this reason our material would seem to be of particular value for the prognosis and natural history of the disease.

The course of PXE is characterized by a high frequency of cardiocerebrovascular complications and, sometimes, by serious gastrointestinal hemorrhage, as exemplified in the above case reports. In spite of these facts the average life span of patients with PXE, as judged from the Swedish material (Table I), does not differ significantly from life expectancy in Sweden. However, the total number of deceased patients in whom the diagnosis of PXE was established is very small. This applies particularly to males. Consequently the possibility of a slight decrease in the average life span of PXE patients cannot be entirely excluded if larger materials were to be investigated. Information on life expectancy of PXE patients is very scanty in the literature, however, one case of early death due to hemorrhagic complications has been reported (40).

With regard to the causes of death, as indicated by death certificates and autopsy reports (cases 1, 2, 3, 6, 8, 9, 10, 11 and 12), the frequency of death from cardiocerebrovascular disease is very high. Contrary to reports on some other materials (40), the Swedish PXE material contains only one case of death from gastrointestinal bleeding (case 3).

Among the major clinical PXE manifestations, those in the skin and eyes were present in all our patients and those of the cardiocerebrovascular system occurred in the majority. Our material also indicates very clearly the order in which the various manifestations appear.

Obviously the first manifestations recognized in our patients were the typical skin lesions, which were observed sometimes in early childhood and in most patients in adolescence or early adulthood. As indicated in Table II, the average age at onset of the skin manifestations, in those cases where first recognition was recorded, was 15 ± 3

years. Probably a more detailed examination at an early age would have revealed the presence of the typical PXE lesions when the patients were much younger since in at least two cases the skin changes were observed in early childhood. It is also a well-known fact that the presence of the skin lesions, at the typical sites, can be confirmed by skin biopsy made before the lesions become actually visible (40).

As described by many investigators (21 25 31) the first skin lesions appeared on the neck and, at later stages, the same changes appeared also in the axillar folds and in the inframammary inguinal femoral, antecubital and popliteal regions. No consequences of the skin lesions, other than cosmetic, were noted.

Involvement of mucous membranes in typical PXE changes was comparatively rare in the present material, and this applies also to changes in the oral cavity (cases 18 and 23) the vagina (cases 24 and 25) or the portio uteri (case 15) Endoscopic studies of the upper gastrointestinal tract, rectum or respiratory tract were not made systematically

Angioid streaks occurred in all our PXE cases, and practically all the patients had severe or significant impairment of vision before death or at the time of our clinical investigation in 1969–71 (Tables III and IV) The impairment of vision was first noted by both patients and physicians, about 20 years after the skin lesions, i.e at a mean age of 43 ± 3 years In eight patients it was impairment of vision that eventually established the correct diagnosis. Although in no patient did the visual disturbance result in complete blindness, a serious decrease in vision occurred in many patients and was considered by them to be the principal complaint and the main cause of their temporary or permanent reduction in working capacity

As previously mentioned, angioid streaks were observed in all our patients. Although not adequately described in all the records, the appearance of this sign preceded by several years the decrease in visual acuity As reported also by Grönblad and other authors (7 23 32) patients with marked decrease in vision showed, in addition to angioid streaks, macular and peripapillary atrophy and different degrees of retinal-pigment proliferation, known as late manifestations of the disease

The manifestations of severe cardiocerebrovascular disease, which appeared in many of the patients with PXE (Tables V and VI) were recognized somewhat later than the eye lesions, since, on an average, the various signs and symptoms became apparent when the patients were between 32 and 63 years. In certain patients, however manifestations of serious cardiovascular disease appeared very early e.g. a female patient (case 24) who had angina pectoris and intermittent claudication when 18 years of age. The same patient had a gangrenous toe ulcer at the age of 32. Such cases must be considered remarkable and very abnormal. They illustrate both the severity and the dissemination of the vascular involvement in this disease.

Surprisingly enough, in spite of the early appearance of angina pectoris and ECG changes indicating coronary insufficiency only one (case 2) of the Swedish patients with PXE is known to have had cardiac infarction. This might be due to a gradual development of the vascular lesions throughout life thus affording good possibilities for the development of functioning collaterals to the coronary arteries.

The manifestations of peripheral vascular disease which are somewhat more frequent than the cardiovascular also show a similar gradual progress. Although they occasionally appear early in life, and despite the fact that signs of advanced vascular disease are very common at the later stages of the disease progress to complete arterial insufficiency of an extremity necessitating reconstructive surgery or amputation, was never recorded in our material. Moreover in other cases reported in the literature, full-blown gangrene of an extremity seems to have been unusual (14 21). This further illustrates the slow progress of the peripheral arterial disease and the favourable conditions in PXE for the development of collaterals.

Gastrointestinal bleeding is a serious but comparatively rare, complication of PXE. In our series 4 out of 26 patients have had complications due to bleeding from the gastrointestinal tract, a frequency apparently similar to that reported for other materials (4 7 14 15 18 21 43).

The number of individual bleeding episodes varied between 1 and 5 In other materials (40) some patients were reported to have had many more bleeding episodes.

In our patients the bleedings were either slight or moderate necessitating blood transfusions on 4

out of 8 occasions; fatal bleeding due to PXE has occurred in one patient (case 4) but this seems to be extremely rare. Such a case was recently reported (40) in a Chinese family with six members with PXE three of them had had many episodes of severe gastrointestinal bleeding. Two further patients had had bleedings from the respiratory tract. A high frequency of complications due to bleeding was found in this Chinese family compared with the relatively low frequency of complications due to bleeding in most of our patients and in other materials (18, 21 25 31 40)

It might therefore be concluded that not only individual but also familial differences exist as regards disposition to such complications.

In one patient (case 27) a direct observation of the possible source of gastric bleeding was made in connection with a surgical exploration of the upper gastrointestinal tract. As described above the gross appearance of the bleeding spot and the results of the microscopic examination of the gastric submucosa revealed the presence of vascular lesions, possibly due to connective tissue degeneration within the vessel wall. These observations are in agreement with those of Kaplan and Hartman (25) and other authors (15, 18, 30)

Pathologic bleedings from other organ systems, e.g. the respiratory and the urogenital tracts, which have occasionally been reported in other materials (5 18) have not occurred in the Swedish patients.

With regard to the possible connection between PXE and the occurrence of diseases in organ systems other than those discussed above only very limited conclusions can be drawn from the sporadic cases of disorders of the endocrine organs, the kidneys, and the nervous system. In our opinion, it seems at present impossible to know whether or not the simultaneous occurrence of PXE and the above-mentioned disorders is merely coincidence or involves some kind of biological relation. Studies of other PXE materials seem also to be more or less inconclusive in this respect (9 19 24 26, 39)

With regard to the psychic disturbances, it has been noted by other authors, including earlier Swedish investigators, (5 7 18 40) that neurasthenia in particular and psychic disorders in general, are common among PXE patients. In our opinion these statements are open to criticism, since careful psychiatric and psychologic explorations of the PXE cases of various materials, including the Swedish cases, ha e not been systematically carried out.

The comprehensive laboratory survey of our PXE patients was unrevealing. No characteristic abnormalities which could be ascribed to PXE were observed in any of the individual biochemical or immunologic blood tests. This also applies to the various urinary screening tests, which included determinations of total hydroxyproline ex cretion.

Table VI shows that for six of the eight patients studied the hydroxyproline excretion was within the normal range whereas two of them had somewhat raised values. According to the few reports in the literature on hydroxyproline excretion in PXE contradictory results have been obtained. Both normal (36) and decreased (28) hydroxyproline excretion have been reported. Since hydroxyproline excretion is considered to reflect collagen breakdown, particularly in the skeleton, it might be concluded, from the results of our study and of earlier in estigations, that ex tensive breakdown of collagen is not a charac teristic feature of PXE.

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HEMODYNAMIC EFFECTS OF METHOXAMINE IN PATIENTS WITH LEFT TO-RIGHT SHUNTS

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Abstract. In nine patients with atrial septal defect (ASD), five with ventricular septal defect (VSD) and one with persistent ductus arteriosus (PDA) hemodynamic examinations have been performed before and after injection of the vasopressor agent methoxamine. On an average 8 mg methoxamine was injected into the pulmonary artery. An insignificant increase of the left-to-right shunt was observed. Significant changes were decrease of heart rate, increase of systemic systolic pressure, mean pulmonary capillary wedge pressure and arteriovenous O_2-difference. Stroke volume and systemic arteriolar resistance increased, and these changes were significant in ASD, insignificant in VSD. The rise in pulmonary arterial mean pressure and oxygen consumption, reduction in cardiac output and pulmonary arteriolar resistance were insignificant.

Methoxamine is a sympathomimetic drug, β-hydroxy-β-(2,5-dimethoxyphenyl)-isopropylamine. It has only a peripheral vasoconstrictor activity without significant inotropic effect upon the ventricular heart muscle (3, 4). It would be of interest to examine the change in left-to-right shunt during different systemic arterial pressures. As methoxamine is a suitable agent to elevate the blood pressure, hemodynamic examinations before and after injection of methoxamine have been performed.

MATERIAL

The material consisted of nine patients, seven women and two men, with atrial septal defect (ASD). Eight had secundum defects and one was of the primum type. Average age was 42 years, varying from 16 to 61 years. Eight of them were operated with closure of the defect, but one of the secundum defects was not operated on owing to pulmonary hypertension. In addition five patients, four men and one woman, with ventricular septal defect (VSD) were examined. Their age varied from 15 to 57 years, average 28 years. So far only one patient with VSD has been operated on. The material also comprised 20-year-old man ith persistent ductus arteriosus (PDA).

METHODS

Right heart catheterization was performed with Cournand catheter. In the cases with VSD and PDA and in six cases with ASD polyethylene catheter was placed by the Seldinger technique in the aorta via the femoral artery. In three cases with ASD thin teflon catheter as introduced into the brachial or femoral artery by modified Seldinger technique. In all patients with ASD during recording of cardiac output, blood samples from the superior and inferior caval veins, the pulmonary artery and the aorta were taken. In five cases the samples from the right side of the heart were taken from the Cournand catheter in the different positions, in four patients from the caval veins via an extra polyethylene catheter which was placed there by the Seldinger technique.

The hemodynamic examinations were performed before and after injection of methoxamine (Vasoxine, B. W. & Co) into the Cournand catheter placed in the pulmonary artery. Methoxamine was diluted in physiologic saline to 1 mg/ml, and 2 mg/min was injected. The injections were stopped when the systemic arterial systolic pressure had been elevated 50 mmHg or more. The dose varied from 2 to 12 mg, on an average 8 mg. Every second minute for at least 10 min the mean value of the pulmonary capillary wedge pressure and the pulmonary artery pressure, further the systemic systolic arterial pressure, were recorded. The cardiac output was estimated according to Fick principle. The volume of expired air was measured with Tissot spirometer, and oxygen consumption was calculated from gas analysis by the micromethod of Scholander. The determination of the cardiac output was performed before and approximately 6 min after start of the injection of methoxamine. The left-to-right shunt was calculated according to the usual formula, expressed both in l/min and in % of the circulation in both the pulmonary and systemic circulation. The pulmonary flow ratio (PFR) as calculated directly as pulmonary blood flow/systemic blood flow. In addition pulmonary arteriolar and systemic arteriolar resistances were calculated.

RESULTS

The data recorded are presented in Tables I and II. The statistical calculations were made separa-

Table I. *Hemodynamic effects of methoxamine in ASD*

		Before		After			
		Mean	S.D	Mean	S.D	t	p
Heart rate (beats/min)	9	79	13.2	61	12.3	6.71	<0.001
Pulmonary capillary wedge pressure (mean, mmHg)	8	3	2.2	6	2.8	5 12	0.001<p<0 005
Pulmonary rterial pressure (mean, mmHg)	9	21	24 1	23	26.1	1.85	—
Systolic systemic pressure (mmHg)	9	131	22.2	171	29 1	5.64	<0 001
Oxygen consumption (ml/min)	9	194	32.6	213	38.4	2.68	—
Arteriovenous oxygen difference (ml/l)	9	44.6	11.6	51.5	12.6	5.06	<0 001
Cardiac output (l/mi)	9	4.5	0.9	4.3	0.9	1.39	—
Stroke volume (ml)	9	57	9.5	70	11.9	5.26	<0.001
L–R shunt (l/min)	9	6.4	3.6	7 8	4 0	1.62	—
L R shunt (% of pulmonary circulation)	9	53	21.0	60	18 0	2 59	—
L–R shunt (% of systemic circulation)	9	146	90.0	181	95.3	1.65	—
Pulmonary flow (l/min)	9	10.8	4 0	11.9	4 7	1.37	—
Pulmonary flow ratio	9	2.4	0.9	2.8	1.0	1.57	—
Pulmonary arteriolar resistance (dynes sec cm^{-5})	8	333	770 6	322	789.3	1 19	—
Systemic arteriolar resistance (dynes sec cm^{-5})	9	1 891	576 8	2 479	621.6	3 75	0.005<p<0 01

tely in the atrial and the ventricular septal defects.

In all the patients a significant decrease of heart rate and increase of the systemic systolic pressure were observed. A significant rise in mean pulmonary capillary pressure was also recorded but the pressure in the pulmonary artery only showed a trend to increase. As both oxygen consumption and arteriovenous oxygen difference increased, the last parameter significantly only insignificant decrease in cardiac output was culated. The stroke volume increased significantly in the ASD group insignificantly in the patients with VSD There was a small increment of the left-to-right shunt after methoxamine, but this was insignificant. As there was a trend to increase of the pulmonary flow there was also a tendency to reduction of the pulmonary arteriolar resistance. On the contrary there was an increase of the systemic arteriolar resistance significant in ASD Two patients had small right to-left shunts. In the case with ASD and pulmonary hypertension the right to-left shunt (0.6 l/min) was almost unchanged (0.7 l/min) after methoxamine. One of the patients with VSD had a right to-left shunt (0 7 l/min) which disappeared after injection of methoxamine.

In the single patient with PDA the changes were principally of the same kind as in ASD and VSD. There was a reduction of the heart rate, increase of the mean pressure in the pulmonary artery systemic systolic pressure oxygen consumption and arteriovenous difference. In this patient cardiac output increased from 5.5 to 6.6 l/min The blood flow in the pulmonary circulation also increased, but there was a small reduction of PFR. As expected, an increase in the systemic arteriolar resistance was observed.

No serious complications were seen. One patient had atrioventricular block and bradycardia for about one minute without feeling unwell. Insignificant side-effects such as transient headache and chilly sensations were observed.

DISCUSSION

Methoxamine exerts its pressor effect both in the dog (3) and in man (4) solely by peripheral vasoconstrictor activity without any significant positive inotropic effect upon ventricular heart muscle. Additionally there has been demonstrated direct depression of the myocardium following intravenous or coronary arterial injection or infusion of larger doses of methoxamine (8 13 14).

As may be expected and found by others (3, 10) injection of the vasopressor agent methoxamine increased the systemic arteriolar resistance, with a significant change in the patients with ASD The systemic systolic pressure increased significantly in both ASD and VSD.

Table II. *Hemodynamic effects of methoxamine in VSD*

		Before		After			
		Mean	S.D	Mean	S.D		p
Heart rate (beats/min)	5	77	7.8	58	13.9	4.86	$0.005 < p < 0.01$
Pulmonary capillary wedge pressure (mean, mmHg)	4	5	2.4	10	3.4	6.33	$0.005 < p < 0.01$
Pulmonary arterial pressure (mean, mmHg)	5	15	6.3	17	8.5	0.71	—
Systolic systemic pressure (mmHg)	5	122	11.9	158	7.3	4.61	$0.005 < p < 0.01$
Oxygen consumption (ml/min)	5	252	37.4	275	67.0	1.77	—
Arteriovenous oxygen difference (ml/l)	5	46.4	5.9	55.1	7.0	5.08	$0.005 < p < 0.01$
Cardiac output (l/min)	5	5.5	1.0	5.1	1.4	1.25	—
Stroke volume (ml)	5	72	16.0	93	35.3	2.17	—
L–R shunt (l/min)	5	6.0	4.0	7.6	5.6	0.73	—
L–R shunt (% of pulmonary circulation)	5	49	18.1	54	21.2	1.16	—
L–R shunt (% of systemic circulation)	5	120	98.6	156	100.7	0.95	—
Pulmonary flow (l/min)	5	11.4	3.6	12.7	6.0	0.56	—
Pulmonary flow ratio	5	2.1	1.0	2.6	1.0	1.09	—
Pulmonary arteriolar resistance (dynes sec cm^{-5})	4	73	34.7	52	47.6	0.89	—
Systemic arteriolar resistance (dynes sec cm^{-5})	4	1413	274.9	1816	408.3	2.43	—

Ueda et al. (12) observed an intensification of the systolic murmur of VSD and the continuous murmur of PDA after injection of methoxamine. They concluded that this was caused by an increased left-to-right shunt. These authors also observed an increase in the pulmonic systolic murmur with the increase in the tricuspid flow murmur in some cases of ASD. The rise in the left atrial pressure may increase the left-to-right atrial shunt in these cases. One may also anticipate that a higher systemic arterial pressure would increase the left-to-right shunts in septal defects. This would especially be logical at ventricular level. In experimental atrial defects, aortico-pulmonary communications and ventricular septal defects in dogs Tanenbaum and Pfaff (11) found an increase in the left-to-right shunt after infusion of methoxamine, but no mention was made as to whether this change was significant or not. In the present material an increase of the shunt was observed in patients with ASD, VSD and PDA, but statistical calculation showed insignificancy both in ASD and VSD. Two patients in this material had small right-to-left shunts, and this direction of the shunt disappeared in one patient with VSD after injection of methoxamine. This change may easily be explained as a result of increased systemic arterial pressure.

A significant decrease of heart rate was recorded as previously found both in dog and man (1, 3, 5, 6, 10). Experiments in dogs have shown that the baroreceptors in the carotid sinuses and aortic arch are responsible for the bradycardiac responses to intravenous injection of methoxamine.

In this investigation there was a significant increase of arteriovenous oxygen difference and an insignificant increase in oxygen consumption. Stanfield and Yu (10) also found an increase in arteriovenous oxygen difference in all patients, but no consistent change in oxygen consumption. In the present material the cardiac output was insignificantly reduced, corresponding with a previous investigation (5) in which no significant change was found. With indicator dilution technique Yu et al. (15) found a significant decrease, while Stanfield and Yu (10) observed a decrease or no change by the Fick procedure. In dogs a decrease in cardiac index in proportion to the decrease in heart rate has been observed (3) whereas the average stroke volume index was maintained at near control values. In the present material a significant increase of the stroke volume was observed.

Methoxamine increases the arterial diastolic pressure by direct arteriolar constrictor action, and afterload is elevated (7). When changes in afterload occur there will be reciprocal changes in cardiac output which need not reflect changes in the myocardial contractile state. An elevation of

afterload will in turn evoke a small increase in preload and the latter will oppose the depression of stroke volume anticipated from the augmented afterload (2) According to these considerations one would expect a small reduction of cardiac output as found in this material.

However methoxamine causes a significant decrease of heart rate and consequently there is also observed a significant increase in stroke volume in the present material.

On account of elevated afterload one would anticipate an increase in the degree of left-to-right shunt. This change was, as mentioned, insignificant.

Both in ASD and VSD a significant rise in the mean pulmonary capillary wedge pressure was found in correspondence with the recorded mean left atrial pressure in dog and man in other investigations (3 9 10)

In the present material there was no significant change in the pulmonary arteriolar resistance. Stanfield and Yu (10) found a significant increment in pulmonary arteriolar resistance in four of six patients with mitral insufficiency but this finding was inconsistent in subjects in the other groups. The same authors observed a significant rise in pulmonary artery mean pressure in patients with mitral insufficiency but in this material with shunt patients there was only an insignificant increase. Methoxamine therefore does not seem to exert any vasoconstrictive effect the pulmonary circulation.

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

IV *An Analysis of the Vibratory Perception Threshold*

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Abstract. The vibratory perception threshold (VPT) has been determined in 85 normal persons and 97 patients with chronic renal failure. In *normal persons* VPT was an exponential function of age. The rate of elevation was faster on the lower than on the upper extremity and faster in males than in females. In the *patient group* as a whole the average deviation from the normal mean VPT adjusted for age and sex, was significantly elevated in the upper and lower extremities. Male patients were significantly more affected than female patients. In females no correlation was present with kidney function and/or age. In males the vibratory perception deteriorated significantly with advancing renal failure, and for comparable degree of renal failure the vibratory perception was more severely affected in old than in young patients. The vibratory perception was more affected on the lower than on the upper extremity and on distal than on proximal test spots. The degree of impairment was roughly correlated with the severity of clinical neuropathy. A significant correlation with the motor conduction velocity (V_m) in the peroneal nerve could be referred to a joint covariation with the kidney function. In the median nerve no correlation was present between VPT and electrophysiological data from sensory nerve conduction studies. Like other clinical components of peripheral neuropathy the diagnostic significance of VPT as an indicator of peripheral nerve dysfunction could be characterized by high specificity and low sensitivity. However, VPT is a quantitative measure and readily applicable for bedside examination, which may prove to be of particular value in longitudinal studies.

In an earlier study (18) impairment of the vibratory perception was shown to be the most frequent clinical sign of peripheral nerve dysfunction in an unselected material of patients with chronic renal failure. However, no detailed account has been published so far on the *vibratory perception threshold* (VPT) in uraemia, although its clinical significance has been much appreciated by several authors, who have studied the peripheral nerve function in diabetic patients (7, 13, 15, 28).

Clinical neuropathy in uraemia is an obstacle, rightly feared, but probably preventable by early and effective haemodialysis, and reversible following early and successful renal transplantation (5, 17). Therefore a reliable and specific quantitative measure of peripheral nerve dysfunction, applicable for a routine bedside examination, would imply considerable advantages in the clinical control of the course of progressive renal failure.

The present investigation was undertaken with the following five objectives:

1. To establish the range of interindividual variation of the VPT in normal persons, considering biological sources of variation due to sex and age.

2. To analyse the degree of VPT impairment in patients with chronic renal failure, and to reexamine the interaction between kidney function, sex and age, which in a previous study (20) appeared to be of decisive importance for the presence or absence of clinical neuropathy.

3. To compare simultaneous VPT determinations on different test spots in order to localize the site of affection on the sensory pathway.

4. To relate the VPT data to other clinical findings and to neurophysiological data in an attempt to evaluate the correlation with the degree of nerve dysfunction and to elucidate the pathophysiology of VPT impairment.

5. To estimate the diagnostic specificity and sensitivity of an elevated VPT value for the detection of peripheral nerve dysfunction in chronic renal failure.

MATERIAL AND METHODS

The control material comprised 85 persons, 41 females and 44 males, from 21 to 65 years of age. All persons were in good health, able to perform their normal work, and a thorough physical examination revealed no signs of acute or chronic illness. Four persons presented a history of a unilateral lumbar disc herniation, and two had

afterload will in turn evoke a small increase in preload, and the latter will oppose the depression of stroke volume anticipated from the augmented afterload (2) According to these considerations one would expect a small reduction of cardiac output as found in this material.

However methoxamine causes a significant decrease of heart rate and consequently there is also observed a significant increase in stroke volume in the present material.

On account of elevated afterload one would anticipate an increase in the degree of left-to-right shunt. This change was, as mentioned, insignificant.

Both in ASD and VSD a significant rise in the mean pulmonary capillary wedge pressure was found, in correspondence with the recorded mean left atrial pressure in dog and man in other investigations (3 9 10).

In the present material there was no significant change in the pulmonary arteriolar resistance. Stanfield and Yu (10) found a significant increment in pulmonary arteriolar resistance in four of six patients with mitral insufficiency but this finding was inconsistent in subjects in the other groups. The same authors observed a significant rise in pulmonary artery mean pressure in patients with mitral insufficiency but in this material with shunt patients there was only an insignificant increase. Methoxamine therefore does not seem to exert any vasoconstrictive effect on the pulmonary circulation.

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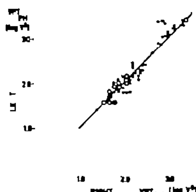


Fig. 1. Comparison between VPT (log V^{μ}) measured on right and left big toe. – – – = ±2 S.D. I the mean difference between the two sides in 85 normal persons. O = Identical observations in 2 to 5 patients.

while the average VPT on the PH and the MM was significantly higher in males than in females. This was not due to differences in the age distribution of the two sexes. VPT values recorded on the lower extremitiy were therefore analysed in females and males separately.

The VPT was significantly correlated with age. The coefficients of the linear regression of VPT (log V^{μ}) on age (years) are given in Table II. The normal range of variation was defined as the age-adjusted mean value $\pm 2 \times S_{yx}$, the standard error of estimate. As indicated by the slope of the regression lines, *b*, VPT increases more rapidly on the lower than on the upper extremity. From the regression equations nearly identical VPT values are obtained in females and males 20–30 years of age. The steeper slope of the regression lines in males, resulting in the sex difference demonstrated above, indicates that the vibratory perception is more susceptible to the ageing process in males than in females.

VPT in Chronic Renal Failure

The average VPT values were significantly higher *in patients with chronic renal failure than in the* control group on all three test spots (Table I). This was not due to differences in age and sex distribution of the two materials.

Fig. 2 shows the VPT values recorded on the PH related to age in patients with chronic renal failure. In order to obtain comparable data, all VPT values recorded in patients were expressed as the deviation, ΔVPT (+/− log V^{μ}) from the age-adjusted mean value as defined by the normal regression equations for females and males. The average ΔVPT values recorded on the three test spots are presented in Table III, which shows that the vibratory perception on the lower extremity independently of age, is significantly more impaired in male than in female patients.

In this material of unselected patients a positive correlation was present between the kidney function (log C_{Cr}) and age (years) of the patients, $r = 0.43$ (females) and 0.52 (males) $p < 0.001$. Both these factors may influence the degree of impairment of the vibratory perception. Hence a 3-variable correlation analysis was applied, where $X_1 = \Delta VPT_{PH}$ (log V^{μ}), X_2 = kidney function (log C_{Cr}) and X_3 = age (years).

In *females* ($n = 46$) ΔVPT_{PH} was not significantly correlated to the kidney function, $r = -0.11$, $p > 0.10$ (Fig. 3) and the 3-variable correlation coefficient, considering also covariation with the age, did not reach a significant level ($R_{1,23} = 0.15$, $p > 0.10$).

In *male* patients ($n = 51$) deterioration of the kidney function was accompanied by a significant rise in ΔVPT_{PH}, $r = -0.46$, $p < 0.001$ (Fig. 3). However, a significant part of the residual variation in ΔVPT_{PH} (i.e. variation not explained by

Table II. *The regression of VPT (Y = log V^{μ}) on age (X = years) in normal persons, 21–65 years old*

Test spot	Sex		$Y = a + bX$		S_{yx}		p
				b			
PP	♀+♂	45	1.1649	0.0072	0.1669	0.4382	<0.005
PH	♀	41	1.2277	0.0149	0.2089	0.6508	<0.001
	♂	44	1.0066	0.0249	0.2325	0.7687	<0.001
MM	♀	41	1.4472	0.0138	0.2224	0.5959	<0.001
	♂	44	1.4454	0.0177	0.2111	0.6864	<0.001

Standard error of estimate.

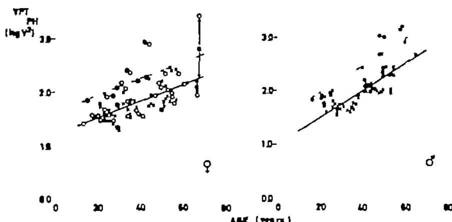


Fig 2 VPT (log V^2) measured on the PH related to age in 85 normal persons ( ) and 97 patients with chronic renal failure (○ = females, ● = males). - - - - range of normal variation in females and males as defined by the regression of VPT on age ±2 S_{yx} (cf. Table II). — the age-adjusted deviation from the normal mean value (ΔVPT = +/− log V^2).

deterioration of the kidney function) could be related to the age of the patients, $r_{13.2} = 0.3442$, $p < 0.025$. The 3-variable correlation equation is given in the legend to Fig. 4 where the estimated relationship between ΔVPT_{PH} and the kidney function is graphically presented for ages of male patients. It is demonstrated that the vibratory perception is better preserved in the younger than in the older age groups for any given degree of renal failure.

Differences between the Recording Sites

In *the control group* VPT was always higher on the PH than on the PP, two structurally comparable test spots, and the difference increased gradually with advancing age ($r = 0.44$, $p < 0.005$, $n = 45$).

The difference was more prominent in *patients with chronic renal failure*, especially in males (Table I). Thus among 95 patients ΔVPT_{PH} was significantly elevated in 34, while ΔVPT_{PP} was elevated in 15 patients, $\chi^2 = 8.91$, $p < 0.01$.

In *the control group* the VPT (in terms of volt age) was generally lower on the PH than on the MM ($p < 0.001$), but in males the difference tended to be eliminated with age, $r = -0.35$, $p < 0.025$, $n = 44$. VPT was highest on the big toe in ten persons (12%), nine of whom were above the age of 40 years, and eight were men.

In *chronic renal failure* ΔVPT_{PH} and ΔVPT_{MM} were significantly elevated in an equal number of patients, 35 and 36 respectively. Thus, from a diagnostic point of view, neither of the two test

Table III. *Mean 24-hour endogenous creatinine clearance (log ml/min) and age-adjusted deviation (Δ VPT = log V^2) from the normal VPT in 97 patients with chronic renal failure*

	Females			Males			
		Mean	S.D		Mean	S.D	Difference between sexes
Creatinine clearance	46	0.8994	(0.5083)	51	0.8567	(0.5012)	$p > 0.30$[b]
ΔVPT							
PP	46	0.0913 $p < 0.01$	(0.1949)	49	0.1485 $p < 0.001$	(0.2092)	$0.10 > p > 0.05$
PH	46	0.2671 $p < 0.001$	(0.4627)	51	0.5021 $p < 0.001$	(0.5108)	$p < 0.02$
MM	46	0.1571 $p < 0.02$	(0.3757)	51	0.4030 $p < 0.001$	(0.3830)	$p < 0.005$

Significance of difference from controls. Also insignificant when $C_{Cr}/1.73$ m² BSA was used.



Fig. 3 Correlation between the VPT expressed as the age adjusted deviation from the normal mean value ($\Delta VPT = \log V^2$ cf. in Fig. 2), and the kidney function ($\log C_{Cr}$) in 46 females and 51 males with chronic renal failure. ----- the 95% range of normal variation. The coefficients of the regression $Y = a - bX$, are shown in the figures.

spots was superior to the other However the vibratory perception was generally more severely affected on the PH than on the MM, the difference between paired observations ($\Delta VPT_{PH} - \Delta VPT_{MM}$) averaging $+0.1020 \log V^2$ ($p < 0.001$). In 26 patients (27%) the actually recorded VPT was higher on the big toe. This was more frequent than in the control material ($\chi^2 = 5.8861$ $p < 0.05$).

Other Neurological Findings

Clinical symptoms and signs. In a previous study (19) it was shown that impairment of the vibration sense was closely correlated with the presence of other clinical signs of peripheral neuropathy However VPT could not replace other clinical criteria, in particular reflex disturbances.

A classification of the neuropathy in order of severity of affection was attempted from the number and distribution of clinical findings, including impairment of the vibration sense. Eight classes were suggested (19). The reliability of this classification was reexamined, calculating the mean ΔVPT_{PH} in each class (Table IV). A steady increase was observed through the eight classes, but the mean values clearly f ll into three groups. In patients without objective findings ($n = 46$), the mean ΔVPT_{PH} came close to zero. In patients who presented a single positive sign ($n = 25$) the mean ΔVPT_{PH} was just abov the upper normal limit but significantly lower than in patients presenting two or more signs ($n = 26$) There was no significant difference between classes within these groups.

Nerve conduction velocity The VPT on the PH and the motor conduction velocity (V_m = m/sec) of the peroneal nerve (capitulum fibulae ankle) were measured on the same extremity in

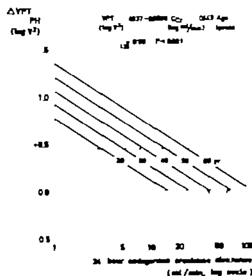


Fig. 4 The estimated relationship between the VPT (ΔVPT_{PH}) and the kidney function (C_{Cr}) for ages of male patients ($n = 51$) with chronic renal failure. The correlation lines were calculated from the 3-variable correlation equation shown in the figure. The partial correlation coefficients, and differed significantly from zero (see text) ---- the upper limit of variation in 44 normal men.

Table IV *Correlation between impairment of the* Δ *VPT*$_{PH}$ (log V^2) *and clinical grading of neuropathy in 97 patients with chronic renal failure*

p = p of difference from the age-adjusted normal mean value; p_b = p of difference between groups

		Mean	S.D	p	p_b
No neuropathy	46	0.0343	(0.2247)	n.s.	
0. No symptoms	16	−0.0112	(0.2545)	n.s.	
I. Cramps/restless legs	20	0.0135	(0.2034)	n.s.	
II. Other symptoms	10	0.1490	(0.1951)	n.s.	
Mild neuropathy	25	0.4924	(0.4064)	< 0.001	< 0.001
III. One sign only	16	0.4650	(0.3714)	< 0.001	
IV. One sign + symptoms	9	0.5411	(0.4823)	< 0.005	
Moderate to severe neuropathy	26	0.9234	(0.4147)	< 0.001	< 0.001
V. Two or more signs, lower or upper extremity	11	0.8536	(0.4294)	< 0.001	
VI–VII. Severe symptoms and signs	15	0.9746	(0.4109)	< 0.001	

49 patients. There was a highly significant correlation between ΔVPT_{PH} and the V_m, $r = -0.5273$ $p < 0.001$ (Fig. 5) but it should be noted that ΔVPT_{PH} was within normal limits in 13 patients in spite of a moderate to severe reduction of the V_m (see next paragraph).

ΔVPT_{PH} (1) and V_m (2) were both significantly

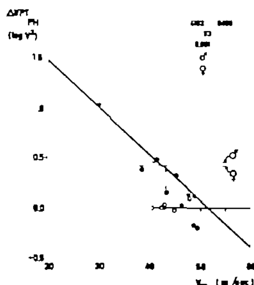


Fig. 5. Relationship between the VPT (ΔVPT_{PH}) and the V_m in the peroneal nerve in 48 patients with chronic renal failure. The regression equation, $Y = -\Delta X$ is given in the figure. -- -- = the upper normal limits of ΔVPT_{PH} (horizontal) and lower normal limit of V_m (vertical). One patient (no. 20, male 24 years) was excluded from the calculation since the V_m could not be determined, as no potential could be evoked from the ext. dig. brev. muscle (ΔVPT_{PH} was +1.80 log V^2).

correlated with the kidney function, C_{Cr} (3): $r_{13} = -0.5477$ $p < 0.001$ and $r_{23} = 0.6590$ $p < 0.001$. Having corrected for covariation with the kidney function, ΔVPT_{PH} and V_m were no longer significantly correlated, as demonstrated by the coefficient of partial determination. $r^2_{12.3} = 0.0702$, $0.10 > p > 0.05$.

In the upper extremity no significant correlation was present between the VPT on the PP and the conduction velocity in the unilateral median nerve, following supramaximal stimulation of sensory fibres in the thumb or between VPT and the amplitude or temporal dispersion of the evoked sensory nerve action potential ($n = 51$).

Diagnostic Significance

The interrelationship between (I) reduced nerve conduction velocity (median and/or peroneal nerve) (II) ΔVPT_{PH} and (III) other clinical signs was calculated in 51 patients. The results are presented in Fig. 6. The *prevalence* of the three components (C) in the diagnostic universe (U), $n(C)/n(U)$ was 0.78 (conduction velocity) 0.45 (ΔVPT_{PH}) and 0.35 (other clinical signs).

No single component fulfils the criteria of an exclusive and exhaustive definition of peripheral neuropathy (S). Considering the broad definition, where S comprises all patients presenting one or more of the three components I, II, III, the *diagnostic specificity* $P(s|c) = n(S \cap C)/n(C)$, of any of the components is $= 1$ ($\cap$ = both/and), implying that the probability of a false negative observation is zero. The *diagnostic sensitivity* of

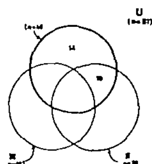


Fig 6. Venn diagram illustrating the interrelationship between components of peripheral neuropathy in 51 patients with moderate to severe renal failure (U = diagnostic universe). I = reduced nerve conduction velocity (peroneal *and/or median nerve*). *II = impaired vibratory perception* (PH). III = other clinical signs. Figures within the circles indicate the number of patients presenting one or more of the components. I nine patients all three components were absent. An analysis of the diagnostic significance of the single components is given in the text and in Table V

the components, $P(s|c) = n(S' \cap C')/n(C')$ (i.e. the probability (P) that the absence of a component (c) indicates the absence of neuropathy (s)) differs considerably. Thus the conduction velocity has a sensitivity of 0.82.

(This is probably a minimum figure due to the restricted diagnostic universe, predominantly comprising patients with severe renal failure. If the diagnostic universe were extended to cover more patients with mild or moderate renal failure, a greater number of patients presenting neither reduced conduction velocity nor neuropathy might be expected, hence increasing the sensitivity of reduced conduction velocity.)

In contrast, the diagnostic sensitivity of impaired vibratory perception or of other clinical signs is very low 0.32 and 0.27 respectively implying that in severe chronic renal failure the absence of these components may give a false prediction of normality in no less than 62 and 73%. For reasons stated above these figures may be expected to represent maxima due to the restricted diagnostic universe.

As shown in Fig. 6, the conclusions reached above remain practically unaltered when a neuropathy is more strictly defined by reduction of the nerve conduction velocity. *The diagnostic specificity* and sensitivity of three components are given in Table V which also includes an evaluation of the diagnostic significance of (IV) *neurological symptoms* as a separate component (not shown in Fig. 6).

The presence of symptoms may illustrate the degree of iatrotropy i.e. the motivation of the patient to seek medical assistance.

Symptoms were present in 23 of the 51 patients, 20 of these had reduced conduction velocity (diagnostic specificity = 20/23 = 0.87). Among 28 patients without symptoms only 8 had normal conduction velocities (diagnostic sensitivity = 8/28 = 0.29)

The diagnostic specificity of symptoms was of the same order of magnitude as that of $\angle VPT_{PH}$ and other clinical signs, indicating that the presence of symptoms should be regarded as a valu-

Table V *Diagnostic significance of components of peripheral neuropathy in 51 patients with chronic renal failure* (*cf* Fig 6)

∩ = both/and, U = diagnostic universe (51 patients), C/C' = component present/absent, S/S' = peripheral neuropathy present/absent

	Prevalence $\frac{(C)}{(U)}$	Diagnostic specificity $\frac{(S \cap C)}{(C)}$	Diagnostic sensitivity $\frac{(S' \cap C')}{(C')}$	Iatrotropy	Accessibility
I. Reduced nerve conduction velocity	0.78	1.00*	1.00*	—	(+)
II. Impaired vibratory perception	0.45	0.96	0.36	—	+
III. Other clinical signs	0.35	0.80	0.27	—	++
IV Symptoms	0.45	0.87	0.29	bad	++

* According to definition.

able indicator of neuropathy. The very low diagnostic sensitivity implies that the presence of peripheral nerve dysfunction remains unnoticed in a great number of patients unless specifically looked for *on the initiative of the doctor*, i.e. the iatrotropy of symptoms is bad.

DISCUSSION

Normal variation of VPT

The results of the present investigation of VPT in normal persons are consistent with the findings of other authors (7, 13, 15, 24, 27). It is demonstrated that VPT is an exponential function of age, which is compatible with the pattern of threshold values of other sensory stimuli (8, 9). The log-normal distribution of VPT values in normal persons was originally demonstrated by Iversen (10) and Mirsky et al. (15). Steiness (27) suggested the logarithmic function, $z = \log(y - 3)$, $y = \text{VPT}$ (in volts), in order to account for an increasing dispersion of values with age. The VPT becomes symmetrically impaired by advancing age irrespective of the test spot, but the rate of increase is significantly faster in the lower extremity, an observation also made by Rosenberg (24). The present material did not show any sex difference in *young* persons, but on the lower extremity the vibratory perception was affected more severely by ageing in males than in females (15, 27), suggesting that the ageing process of *the sensory nerve system may be subject to biological variation according to sex*. This implies that VPT in patients should be related to normal values in the corresponding sex.

When determined with the technique used in the present study, nearly all previously reported VPT values in normal persons fall within the limits of normal variation as defined in Table II (7, 13, 15, 26, 27). Below the age of 50 years normal values rarely exceed 20 volts ($= 400\ V^2$), which is less than one sixth of the maximal stimulus strength (50 volts $= 2\,500\ V^2$), since the stimulus strength is a function of the square of the voltage (23). In another study (22) the reproducibility of repeated determination several days apart was shown to be independent of the stimulus strength when expressed on a logarithmic scale, amounting to 50–60% of the interindividual variation.

VPT in uraemia

A significant elevation of the VPT is demonstrated in patients with chronic renal failure. It is noteworthy that the deviation from the normal values is not a simple function of the degree of renal failure. Although adjusted for normal variations due to sex and age, these two factors still appear to play a significant role for the degree of impairment. Thus the vibration sense in females of all ages and in the younger age classes of male patients was less frequently and less severely affected by comparable degree of renal failure. The quantitative analysis of a single component of peripheral neuropathy therefore suggests that the premorbid state of the peripheral nerve system significantly modifies the effects of uraemic intoxication. This conclusion is compatible with the results obtained in a previous analysis of the total clinical picture of neuropathy in chronic renal failure (20). The lack of correlation between ΔVPT and the kidney function (C_{Cr}) in females is a surprising feature, also present when C_{Cr} was corrected to a body surface area of 1.73 m². Replacing the C_{Cr} by the serum creatinine concentration as an index of the degree of azotaemia did not change the result. The fact that renal failure was more pronounced in young than in older females may have obscured the correlation, although no significant improvement was obtained from a multivariate correlation analysis including the age of the patients. This may partly be due to the limited number of observations, but it cannot be excluded that the heterogeneity of the material (primary renal disease, duration of azotaemia, complicating disorders, etc.) may include overlooked factors of importance for the VPT.

Recently it has been demonstrated that VPT parallels the clinical improvement and deterioration in the course of long-term intermittent haemodialysis in patients with terminal renal failure (14). This compares well with the rapid normalization of the VPT following renal transplantation, *observed by the present author* (17). These observations clearly indicate a direct relationship between the peripheral nerve function and uraemic intoxication.

Pathophysiology

Theoretically any part of the tract, from the receptor via the peripheral nerve, the dorsal

column to the sensory cortex, may be affected when the vibratory perception is impaired. The fact that VPT on the PP was normal or only slightly affected in spite of pronounced elevation of the VPT on the lower extremity does not indicate an affection of the central part of the tract. For the same reason it is not conceivable that the results have been much distorted by psychological factors unrelated to peripheral neuropathy e.g. lack of cooperation, mental distraction due to uraemic intoxication etc. The acuity of vibratory perception on the MM and the PH cannot be directly compared from the VPT in terms of the voltage owing to characteristic differences in the visco-elastic properties of the two test spots (23) In the patient material, however the VPT on the big toe was *relatively* more impaired than on the malleolus, and in normal males the same tendency was also apparent as a result of ageing. Furthermore serial determinations in young patients show that inversion of the ratio VPT_{MM}/VPT_{PH} normally >1 may sometimes be the earliest clinical indication of the development of peripheral neuropathy (22) indicating a primary and predominant affection of the distal part of the tract, i.e. of the receptor and/or the peripheral nerve fibre. Thus the concept that impairment of the vibration sense is a typical indicator of medullary lesion cannot be maintained.

As emphasized by Calne and Pallis (1) considerable electrophysiological evidence, reported since 1949 (25) supports the early suggestion by Tait (29) that the Pacinian corpuscles are the most important, although not the only potential, receptors responsible for the perception of vibrations. The Pacinian corpuscle is the peripheral end organ of a thick, myelinated afferent nerve fibre. Histopathological studies of the Pacinian corpuscles in uraemic patients are still lacking, but Cauna and Mannan (2) demonstrated that the Pacinian corpuscles were subject to regressive changes with age. This has been suggested as a possible explanation for the increasing vibratory perception thresholds in old persons (1). In the diabetic literature (13 15 28) impairment of VPT is commonly related to ischaemic changes due to microangiopathy of the vasa nervorum, hypothesis which has been much challenged by other authors (30). Gilliatt and Willison (6) advanced the hypothesis that impairment of the VPT was due to temporal dispersion of the pattern of afferent impulses resulting from segmental demyelinization of the afferent nerve fibres. The latter has been demonstrated in histopathological studies in diabetics (30) as well as in uraemic patients (4 12), and increased temporal dispersion of the evoked sensory nerve action potential is a characteristic finding in these patients (16)

Gregersen (7) demonstrated a statistically significant correlation between VPT_{PH} and the motor conduction velocity in the peroneal nerve in young diabetic patients. The same relationship was observed in the present material. However both variables were significantly correlated with the kidney function, and a relationship independent of this common source of variation could not be demonstrated. Longitudinal studies further indicate that elevation of the VPT lags considerably behind the reduction of the conduction velocity (22) Vice versa, the return to normal levels of the VPT following a renal transplantation occurs before normalization of the conduction velocities (17) Any functional relationship between the V_m and VPT can be excluded in advance but in the median nerve impairment of the V_m can be shown to parallel that in sensory fibres. However in the upper extremity *direct comparison* did not reveal any correlation between the VPT on the thumb and the conduction velocity in afferent fibres *of the same nerve*. The fact that VPT on the thumb varies independently of the amplitude and temporal dispersion of the sensory nerve action potential, recorded at the wrist, does not disprove the hypothesis of Gilliatt and Willison (6) since it may be objected that sensory *threshold* stimuli cannot be compared with the pattern of potentials evoked by *supramaximal* stimuli. Thus the pathophysiology of impaired vibratory perception still remains obscure.

Diagnostic significance

In ΔVPT_{PH} a numerical factor is obtained which roughly parallels the clinical severity of peripheral nerve dysfunction. The present study suggests that a clinical grading of neuropathy should be restricted to the following two groups: mild neuropathy and moderate to severe neuropathy" A more detailed classification was not suggested from the VPT data (Table IV).

An analysis of the diagnostic significance of

impaired vibratory perception has not been attempted in previous reports. This investigation demonstrates that a significant elevation of ΔVPT_{PH} is highly specific for the presence of peripheral neuropathy in advanced renal failure, while a normal threshold is diagnostically insignificant, since a reduction of the conduction velocity in peripheral nerves can be expected in more than 50% of the patients. Hence the diagnostic sensitivity is low and no better than that obtained for other clinical components of peripheral neuropathy. The advantage of VPT determinations, however, should be sought among other qualities. The most important advantage is the fact that VPT is a *quantitative* measure of the peripheral sensory nerve function. Moreover, the determination of VPT is easily done as a *bedside* examination and harmless to the patient. The normal variation as well as the reproducibility can be properly defined, and elevated values are relevant and sufficiently specific for peripheral nerve function. The method therefore may be considered a valuable supplement to other diagnostic measures and may prove to be particularly suited for longitudinal studies.

ACKNOWLEDGEMENT

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BLOOD PRESSURE IN SWEDISH BOYS OF 18

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Abstract. In 1965 in connection with enlistment for compulsory military service, 1 789 18-year-old men from practically the whole of Sweden have been examined in respect of different medical variables, e.g. blood pressure (BP), heart rate (HR), body weight (b.wt.). The subjects constituted representative sample of about 3% of the whole population of 18-year-old males. The variation of the sample in systolic and diastolic BP is reported. Due to illness or other causes of decreased fitness for service 394 individuals have been excluded as not being suitable in normal material. For another 80 individuals the examinations were not complete for all variables, and these 80 individuals were therefore also excluded. The remaining material consisted of 1 315 men. Relationships between BP and other variables are presented. The difference in BP between the excluded 474 individuals and the remaining material is small. Multiple correlation and regression between BP (systolic and diastolic), on the one hand, and resting HR and b.wt., on the other are presented.

From 1969 a new system for enlistment of conscripts has been used in Sweden. The main part of the investigation is medical. On the basis of results of the investigations the examined 18-year-old boys are classified in respect of physical functional capacity as well as incapacity due to disease (8).

In the autumn of 1965 a representative normal material from the whole of Sweden was collected. From this investigation the results of the recordings of BP are presented, as well as some of the other medical variables of interest which correlated with BP

Our material is very homogenous judged by criteria such as sex and age. Due to the consistent random selection from the whole of Sweden (except Halland and Gotland) and the relatively large number of participants, we look upon the results as having a certain practical value.

METHODS

Blood pressure was examined by auscultation on the right upper arm with cuff coupled to mercury manometer. The rubber cuff of the manometer was 12 cm broad and 23 cm long. The examination was performed with the subject in the supine position. Before the examination the subjects rested on bed for at least 15 min, together with other subjects in the room. Noise and unnecessary talk were avoided. The room temperature was kept at about 24°C, so that no one should be cold.

The aim of the examination and the procedure had been explained to the subjects earlier. Most of the BPs were recorded by nurse, the rest by an assistant. They were specially trained in the technique of measuring BP. To get standardized examination conditions their work was continuously supervised by two of the authors (Nordgren and Nordesjö) who were also members of the team which visited eight places in Sweden to perform the examination.

Measurement of BP was done in accordance with the recommendation of WHO (15). During the deflation of the cuff the point of first appearance of an audible pulse beat was recorded as the systolic pressure. The diastolic pressure was recorded as the point when the sounds became muffled (phase 4). As rule the point when the sounds disappeared (phase 5) was near the point of muffling (phase 4).

Heart rate (beats min). ECG was recorded immediately before the BP recording. The calculation of the HR was based on at least 15 heart cycles.

Body dimensions. Chest circumference (cm) was recorded at mamillary height after normal expiration, the subjects standing relaxed with the arms hanging. *Waist circumference* (cm) was measured immediately above the crista iliaca under the same conditions. *Body weight* was recorded without clothes and is reported to the nearest 0.5 kg.

The *maximal isometric muscle strength* (kp) of hand

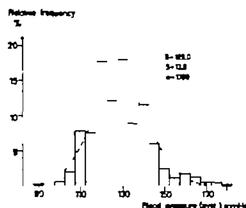


Fig 1 Relative frequency of systolic BP is the Gaussian curve corresponding to the staple diagram.

grip, elbow flexion and knee extension was recorded by the method described by Tornvall (13).

The *vital capacity* (2) was measured with an Expirograph (Godhard) and peak expiratory flow (l/min) with a Wright peak flow meter.

Haemoglobin concentration (g/100 ml) was determined in capillary samples of finger blood, taken with the subject in the sitting position. The analysis was made according to the oxygen haemoglobin method with a Linson Junior Kolorimeter (7).

Heart volume (ml) was calculated from the frontal and lateral (7 7 cm) chest films with the subject standing (6).

MATERIAL

The material consisted of 1 789 18-year-old men randomly selected from all Sweden (except Halland and Gotland) for examination in 1965 in connection with enlistment for military service. The sample constitutes about 3% of the whole enlistment population.

In order to examine the relationships between BP and other variables the material has been reduced. At examination all subjects were classified on the basis of presence of diseases or defects into four categories of military usefulness. 394 individuals looked upon as being of limited military usefulness, and therefore not normal in all respects, were excluded from the material. Furthermore some of the subjects had incomplete data. Therefore, when computing the material, it has been necessary to exclude another 80 young men, not classified as of low military usefulness. Reported relationships consequently refer to 1 315 individuals.

The mean difference between the 474 excluded individuals and the remaining 1 315 is for systolic BP 3.7 mmHg (131.7 mmHg – 128 0 mmHg, $p < 0.001$), diastolic BP 2.0 mmHg (80.6 – 78.6, $p < 0.001$), resting HR 3.9 beats/min (77.8 – 73.9 beats min, $p < 0.001$), b.wt. –0.5 kg (66 0 – 66.5 kg, $p > 0..$). Differences between the groups have consequently been found, except for b.wt., but are small on percentage basis.

The exact mean age has not been calculated. All the subjects were, however, born in the same year (1947). Thus the variation of age is very small.

RESULTS

To make the results easy to survey they have to a large extent been graphically plotted.

Fig. 1 shows the systolic BP and Fig. 2 the diastolic BP expressed as relative frequency in all 1 789 individuals, that is, including subjects not looked upon as being normal in all respects.

The material has a mean systolic BP of 129 0 mmHg $S = 13\ 0$ mmHg, and mean systolic BP 79.2 mmHg, $S = 10.5$ mmHg.

The relationship between diastolic and systolic BP (Fig. 3) is based on 1 315 individuals, as are also the following relationships.

With diastolic BP as a dependent variable a linear equation of regression is obtained.

$$y = 0.352\,x + 33.6 \qquad S = 8.7 \quad r = 0\,46 \tag{1}$$

With systolic BP as a dependent variable the equation of regression is:

$$y = 0\,601\,x + 80\,7 \qquad S = 11\,4 \quad r = 0.46 \tag{2}$$

The dotted lines (Figs. 3 4 and 5) are the 95% confidence interval for a new observation (15).

Both the systolic (Fig. 4) and the diastolic BP (Fig. 5) have been related to resting HR.

The linear equation of regression for the relationship between systolic BP and resting HR is:

$$y = 0.311\,x + 105.0 \qquad S = 12.6 \quad r = 0.29\ (p < 0\,001) \tag{3}$$

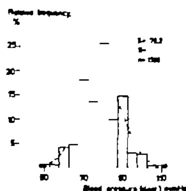


Fig. 2 Relative frequency of diastolic BP is the Gaussian curve corresponding to the staple diagram.

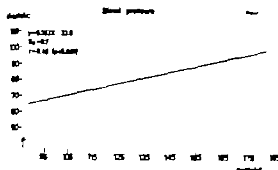


Fig. 3. Relation between diastolic and systolic BP

The corresponding equation of regression for diastolic BP to resting HR is:

$$y = 0.164x + 66.5$$
$$S_y = 9.6 \quad r = 0.20 \ (p < 0.001) \tag{4}$$

From the figure it is seen that the higher the HR the higher the BP and this is more pronounced for systolic BP.

The BP has also been correlated to other variables (Table I). Because both b.wt. and resting HR are related to the systolic BP but the coefficient of correlation between them was 0.01 there is a presumption of a multiple correlation between systolic BP on the one hand, and resting HR and b.wt. on the other. This multiple coefficient of correlation was numerically higher than the corresponding single value. The following multiple relationship was obtained.

$$y = 0.410x + 0.309x + 77.8 \tag{5}$$

where y is systolic BP, x_1 b.wt. and x_2 resting HR.

$S = 11.8 \quad r = 0.39$

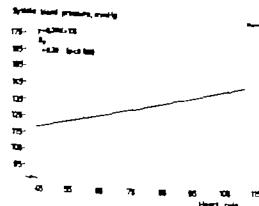


Fig. 4. Relation between systolic BP and HR.

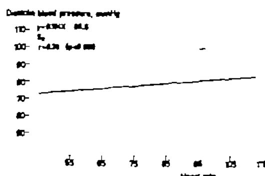


Fig. 5. Relation between diastolic BP and HR.

The multiple coefficient of correlation ($r = 0.39$) is significantly higher ($p < 0.01$) than the single coefficient of correlation between systolic BP and resting HR (eq. 3 $r = 0.29$).

A multiple correlation between diastolic BP on the one hand, and resting HR and b.wt. on the other gave:

$$y = 0.221x_1 + 0.163x_2 + 51.9 \tag{6}$$

where x_1 is b.wt and x_2 resting HR.

$S = 9.5 \quad r = 0.27$

Table I. *Product moment correlations between systolic and diastolic BP and heart volume, anthropometric data, muscle strength, pulmonary function and Hb concentration*

−1315 ≥0.09 $p < 0.001$ 0.05, $p < 0.01$

	BP (mmHg)	
	Systolic	Diastolic
Heart volume	0.18	0.08
Anthropometric data		
B. wt.	0.26	0.19
B. ht	0.05	0.06
BSA (Dubois)	0.22	0.16
Femoral condyle breadth (right side)	0.09	0.11
Radioulnar breadth (right side)	0.08	0.08
Chest circumference	0.23	0.14
Waist circumference	0.26	0.16
Muscular strength		
Hand grip (right side)	0.07	0.07
Elbow flexion (right side)	0.13	0.14
Knee extension (right side)	0.11	0.10
Pulmonary function		
Vital capacity	0.10	0.10
Maximal peak expiratory flow	0.09	0.05
Hb concentration	0.10	0.11

Table II. *Multiple regression between systolic BP on the one hand and, HR and b.wt. on the other*

Means ($\bar{x}$) for systolic BP given for different b.wts. and HRs

HR	B.wt. 45	50	55	60	65	70	75	80	85	90	95	100	105	110	115
45	110	112	114	116	118	120	123	125	127	129	131	133	135	137	139
50	112	114	116	118	120	122	124	126	128	130	132	134	136	138	140
55	113	115	117	119	122	124	126	128	130	132	134	136	138	140	142
60	115	117	119	121	123	125	127	129	131	133	135	137	139	142	144
65	116	118	120	123	125	127	129	131	133	135	137	139	141	143	145
70	118	120	122	124	126	128	130	132	134	136	138	141	143	145	147
75	119	122	124	126	128	130	132	134	136	138	140	142	144	146	148
80	121	123	125	127	129	131	133	135	137	139	142	144	146	148	150
85	123	125	127	129	131	133	135	137	139	141	143	145	147	149	151
90	124	126	128	130	132	134	136	138	141	143	145	147	149	151	153
95	126	128	130	132	134	136	138	140	142	144	146	148	150	152	154
100	127	129	131	133	135	137	140	142	144	146	148	150	152	154	156
105	129	131	133	135	137	139	141	143	145	147	149	151	153	155	157
110	130	132	134	136	138	141	143	145	147	149	151	153	155	157	159

For the diastolic BP the multiple coefficient of correlation is probably significantly higher ($p < 0.05$) than the single correlation between diastolic BP and resting HR (eq. 4, $r = 0.20$).

To survey the multiple relationships between systolic and diastolic BP on the one hand, and b.wt. and resting HR on the other, the mean values for systolic (Table II) and diastolic (Table III) BP have been calculated for different body weights and heart rates.

From Table I it will be seen that b.wt. has a higher correlation than body height (b.ht.) (and] surface) to BP and that chest circumference and waist circumference have a high coefficient of correlation to b.ht. ($r = 0.81$) which indicates that an indirect correlation between BP and these body volume measurements exists. The remaining reported variables in Table I all show low correlations to BP.

DISCUSSION

Blood pressure recording indirectly with a cuff on the upper arm has been analysed from different aspects. Different dimensions as to length and width of the rubber bladder have been recom-

Table III. *Multiple regression between, on the one hand, diastolic BP and, b.wt. and HR on the other*

Means ($\bar{x}$) for diastolic BP given for different b.wts. and HRs

HR	B.wt. 45	50	55	60	65	70	75	80	85	90	95	100	105	110	115
45	69	70	71	72	74	75	76	77	78	79	80	81	82	84	85
50	70	71	72	73	74	76	77	78	79	80	81	82	83	84	85
55	71	72	73	74	75	76	77	79	80	81	82	83	84	85	86
60	72	73	74	75	76	77	78	79	80	82	83	84	85	86	87
65	72	74	75	76	77	78	79	80	81	82	83	85	86	87	88
70	73	74	75	77	78	79	80	81	82	83	84	85	86	88	89
75	74	75	76	77	78	80	81	82	83	84	85	86	87	88	90
80	75	76	77	78	79	80	82	83	84	85	86	87	88	89	90
85	76	77	78	79	80	81	82	83	85	86	87	88	89	90	91
90	77	78	79	80	81	82	83	84	85	86	88	89	90	91	92
95	77	78	80	81	82	83	84	85	86	87	88	89	91	92	93
100	78	79	80	81	83	84	85	86	87	88	89	90	91	9	94
105	79	80	81	82	83	84	86	87	88	89	90	91	92	93	94
110	80	81	82	83	84	85	86	87	89	90	91	92	93	94	95

mended, aiming at as little discrepancy as possible between direct and indirect BP recordings. Different recommendations have been issued. In the present investigation the recommendations of WHO (15) and Tibblin (12) have been followed.

In 1966 Karlefors et al. (5) showed that direct diastolic BP via a catheter in brachial artery in comparison with BP via a cuff (dimension of rubber bladder 10.5×30 cm) phase 5 was 11.3 mmHg lower. There was no significant difference for systolic pressure. Varying arm circumference did not influence the results of their study in respect of systolic pressure. The indirectly recorded diastolic pressure, on the other hand, was overestimated by 15 mmHg on an average in persons with an arm circumference more than 30 cm. Persons with arm circumference less than 26 cm were overestimated by a mean value of 7.5 mmHg for diastolic pressure. The coefficient of correlation between auscultatory systolic pressure and intra-arterially recorded systolic pressure was high ($r=0.89$). This means that indirectly recorded systolic BP did not deviate fundamentally from intra-arterial measurements, while in some persons there may be a not negligible error in recording of the diastolic pressure.

The coefficients of correlation in the present study may be looked upon as low. The material is, however, very homogenous as to age and sex. That is, the correlations should be compared with partial correlation coefficients where the influence of age and sex has been eliminated.

Tibblin (12) examined arm circumference in 96 consecutive subjects. He found that, when using Pickering's table of corrections (11) (for BP measurement with cuff and different arm circumferences), this had little or no effect as regards the majority of the subjects.

Humerfelt (3) and Tibblin (12) did not correct for arm circumference- neither did we. Chiang et al. (1) also conclude in their review: Correcting blood pressure by arm circumference will obscure the important influence of body weight on blood pressure in epidemiological studies.

For clinical routine examinations, especially when the time is limited, as for instance in mass examinations, the indirect BP recording is the only practical method. The clinical evaluation has to be made on the basis of the indirectly measured BP because arterial catheterization demands large resources, including time.

Increase of arterial BP predisposes to several serious diseases, for instance coronary insufficiency (4). Life expectancy has been shown to be correlated to BP. Using statistics from American and Canadian life insurance companies, covering 3.9 million individuals and 102 000 fatal cases between 15 and 69 years of age, Gubner (2) showed that mortality rises with increasing BP. Already at a systolic pressure of 128–137 mmHg the mortality was 118% of the expected. For a diastolic pressure of 83–87 mmHg the corresponding figure was 129%. Measurement of the BP is consequently of practical importance both for the individual and when studying the occurrence of disease and mortality in the population.

Practical, economical and organizing problems usually make it impossible to get a statistically representative sample from a large geographical area. Due to the consistency of sampling from one age group of the boys enlisting for compulsory military service, we had unique means of describing a Swedish population. Our aim is to follow up the results in a longitudinal study after a convenient period, as for instance in the study by Oberman et al. (10).

As will be seen from the staple diagrams (Figs. 1 and 2) there is an uneven distribution with multiples of ten occurring more often than multiples of five. This multimodal type of distribution has been described in several earlier reports on BP measurement, probably due to a tendency of the investigator to prefer multiples of ten. In this respect our results seem to be in analogy with, for instance, the finding by Wallis & Roberts (14) that, when a big population was asked about their age, the answers representing multiples of five and ten were overrepresented. The deviation from the normal variation with an uneven distribution, the mode of the frequency curve being numerically lower than the arithmetical mean, is in analogy with earlier studies (3, 12).

Tables II and III show the simultaneous variation between BP (systolic and diastolic) and resting HR and b.wt. in the material. Because there is no correlation between HR and b.wt. (-0.01) two different factors must exist, one of which shows the correlation between BP and HR and the other the relationship between BP and b.wt. By using a measure of deviation (S_y) in equations 5 and 6 and in Tables II and III the material can be used as a reference material for

BP measurements. B.wt. and HR were the two variables which gave the highest multiple coefficient of correlation to BP. Chiang et al. (1) consider the relationship overweight-hypertension in their review. In several reports BP has been correlated either to b.wt. or HR.

The present study proves that both weight and heart rate should be included when evaluating blood pressure. The remaining variables presented in Table I apart from HR and b.wt. do not explain the variation of BP in the material. Consequently neither measurements of skeletal growth (for instance femoral condylar breadth, radioulnar breadth, body height) nor muscle strength have shown any considerable correlation to the results of the BP measurements in our material.

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DIURNAL VARIATIONS IN THE CONCENTRATIONS OF BLOOD ACETOACETATE, 3-HYDROXYBUTYRATE AND GLUCOSE IN NORMAL PERSONS

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Abstract. The blood concentration of acetoacetate (AA), 3-hydroxybutyrate (3-HB) and glucose has been determined over 24-hour period in 16 normal subjects, 22–39 years old. The mean fasting concentration of AA was 29 ± 10 μmol/l (S.D.). The mean fasting concentration of 3-HB was 38 ± 31 μmol/l (S.D.). During the evening there was characteristic rise in the concentration of AA and 3-HB to peak at midnight, where mean concentrations were 4 times greater than mean fasting concentrations. In most of the subjects ketone body peak was also found at 5 p.m. and in seven also at noon. In connection with the rise in blood glucose following lunch and dinner an inverse relationship was seen between the concentrations of blood ketone bodies and blood glucose. There was considerable positive correlation between blood AA and blood 3-HB

The development of specific, quick and sensitive enzymatic micro-methods for determination of acetoacetate (AA) and 3-hydroxybutyrate (3-HB) has made determination of the blood concentration of these substances as easy as the routine determination of blood glucose. Thus it is now possible to make clinical use of ketone body determinations. In the control of diabetics, for example determination of the ketone body concentration in blood at intervals during a 24-hour period may be of importance. If such determinations are to have clinical use, it is necessary to know the diurnal variations in normal persons unde standardized conditions. We ha xamined, therefore, the concentrations of AA and 3-HB in blood at short intervals during a 24-hour period in normal persons. Since a connection between variations in blood ketone bodies and blood glucose is probable, determinations of blood glucose were performed simultaneously

MATERIAL AND METHODS

In 9 women and 7 men, aged 22 39 years (mean age 31 years), admitted to hospital because of minor diseases of the locomotor system or neurotic disorders, determinations of AA, 3-HB and glucose in capillary blood ere performed 13 times during 4-hour period. None were diabetics or overweight. All the women had regular menstrual periods. During the investigation the subjects were confined to the ward, activity was otherwise unrestricted. They enjoyed an afternoon nap from 1.30–1.30 p.m. and retired for the evening at 9 p.m. They got breakfast at 8 m. (about 56 g carbohydrate, 28 g protein and 25 g lipid), lunch at 12 noon (about 104 g carbohydrate, 23 g protein and 17 g lipid) and dinner at 5 p.m. (about 57 g carbohydrate, 27 g protein and 21 g lipid), totalling about 1 800 calories. Only w ter was given between meals. Blood samples ere taken at 8 a.m., 9.30 m 11 a.m., 12 noon, 2 p.m., 4 p.m., 5 p.m., 7 p.m., 9 p.m. 11 p.m. 1 a.m., 4.30 m. and at 8 m. The 3 blood samples in relation to the meals were taken immediately before the beginning of the meal. For the determination of AA and 3-HB in blood, an enzymatic micro-method was used (6). Blood glucose was determined by an o-toluidine method

RESULTS

Acetoacetate and 3-hydroxybutyrat

Blood concentrations of AA and 3-HB at the various times of sampling are illustrated in Fig. 1

At the start of the 24-hour period of investigation the mean concentration of AA after over night fasting was 29 ± 10 μmol/l (S.D.) the mean concentration of 3-HB 38 ± 31 μmol/l (S.D.). The highest AA as well as 3-HB mean concentration was found at 11 p.m., 113 ± 43 μmol/l (S.D.) and 153 ± 88 μmol/l (S.D.) respectively. The highest AA concentration was 209 μmol/l, the highest 3-HB concentration 337 μmol/l.

The rise in blood concentration of AA and 3-HB during the evening corresponded to a fall in physical activity in the subjects. It is possible, therefore that this concentration peak, entirely or partly was due to post-exercise ketosis, the so-called Courtice Douglas effect (2)

The greater scatter between the concentrations and the tendency to a higher ketone body concentration at the end of the investigation compared with the beginning were probably both due to an increased lipolysis owing to an increasing situation of stress caused by the investigative procedure.

Many factors influence the regulation of the ketone body concentration in the blood during normal circumstances. These factors are still partly unknown and require further investigation.

ACKNOWLEDGEMENTS

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HODGKIN'S DISEASE ASSOCIATED WITH THE NEPHROTIC SYNDROME WITHOUT KIDNEY LESION

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Abstract. Three patients are reported in whom Hodgkin disease was associated with the nephrotic syndrome. Biopsy studies by light and electron microscopy revealed minimal or absent renal lesions, and renal vein obstruction was excluded by angiographic examinations. I tw cases development and remission of nephrotic syndrome was closely related to the course of Hodgkin's disease, while in one patient both conditions remained unrelieved. The close time relationship observed in these patients, as well as in similar cases previously reported, suggests that these two conditions when associated may share common pathogenetic mechanism.

Since 1939 23 cases of Hodgkin's disease associated with the nephrotic syndrome have been reported (1 2, 4 5 9 10, 11 13, 14 15) Considering that both conditions are relatively rare, the possibility of a causal relationship between the two diseases exists. Conceivably the nephrotic syndrome in patients with Hodgkin's disease could be caused by amyloidosis in the kidneys or by renal vein obstruction due to external pressure by enlarged lymph nodes.

We shall report on three cases of Hodgkin's disease associated with the nephrotic syndrome in which the two mentioned possibilities could be excluded by angiographic and kidney biopsy studies.

CASE REPORTS

Case 1

An 18-year-old male ho in May 1968 was admitted to another hospital with nephrotic syndrome which had developed during one week. As chest X-ray revealed tumor in the right side of the upper mediastinum, the patient was transferred to the Department of Thoracic Surgery in this hospital (Fig. 1). At mediastinoscopy bronchoscopy and esophagoscopy there ere no signs of invasive growth into or compression of mediastinal structures, and no enlarged mediastinal lymph nodes were seen. A biopsy from the tumor showed necrotizing granulomatosis which could not be classified. Because of universal edema and severe proteinuria the patient was transferred to the Medical Department C (Table I).

Kidney function was characterized by an increased glomerular filtration rate (Table II) Serum creatinine was 0.6 mg/100 ml, there was continuous proteinuria varying from 2.0–5.7 g/24 h. Urine electrophoresis revealed that 35–100% of the excreted protein was albumin. The serum albumin concentration was extremely low below 0.1 g/100 ml. The α_2-globulin concentration and the γ-globulin concentration were increased (Fig. 2). On serum immunoelectrophoresis an increase of γ G, γ A and γ M was seen.

U rography showed normal kidneys apart from double left ureter. Phlebography of the renal veins did not demonstrate any vidence of compression or thrombosis. X-ray examination of the inferior vena cava showed normal vessel with free passage of contrast medium to the right atrium. Pressure measurements in the inferior vena cava showed gradient between the right atrium and point below the renal veins of 4 mmHg in the upright position and 3 mmHg in the supine position. Lymphography showed normal retroperitoneal lymph nodes, no contrast was seen in the tumor, the thoracic duct had diameter of 8 mm and no sign of compression was seen.

With bed rest the edema decreased and a weight loss of 10 kg took place during 10 days. On ambulation there was weight gain of 3 kg. Treatment with diuretics resulted in disappearance of the edema, but the other abnormalities indicative of the nephrotic syndrome were still present.

On Aug. 23, 1968, the tumor was removed by extirpation. The tumor was adherent to the superior vena cava and the esophagus. The tumor was removed radically. Postoperatively cobalt treatment was given against abdominal and thoracic fields, total of 3 700 rad against each field.

Pathology. The surgical specimen consisted of an encapsulated, firm and whitish tumor weighing
Microscopically the tumor was classified as
matous thymoma which according to recent lev
(3, 6) is to be considered as Hodg se
the thymus.

d



Fig. 1 Case 1. X-ray of the chest. A tumor in the right part of the upper mediastinum is seen.

Table I *Initial findings in patients with Hodgkin's disease associated with the nephrotic syndrome*

Pat. no.	Sex	Age (y)	Proteinuria (g/24 h)	Creatinine clearance (ml/min)	Blood pressure (mmHg)	Se-cholesterol (mg/100 ml)	Peripheral lymph nodes	Spleen/liver
1		18	2.0–5.7	130	170/80	375	Not palpable	Not palpable
2		17	3.2–17.4	120	115/75	325	Palpable	Not palpable
3		53	3.1–14.0	60	140/90	413	Palpable	Not palpable

Table II *Kidney function studies in patient 1*

^{51}Cr ... ^{125}I-iothalamate, ^{131}I-h..., ^{131}I-hippuran

Year	Date	C_{Th}	$C_{Th_{125}}$	C_{IH}	$C_{IH_{131I}}$	C_{Th}/C_{IH} · 100	$C_{Th_{125}}/C_{IH_{131I}}$ · 100
1968	Jul. 22	178	188				
	Aug. 20	146	153				
	Aug. [illegible]3	E[illegible]tirpation of the tumor					
	Sept. 7	189	178	907	894	20.83	19.91
	Sept. 30	Cobalt treatment (finished Nov. 29 1968)					
	Oct. 8	170	176	603	636	28.19	27.67
1969	June 12	124	125	442	453	28.05	27.59
	Oct. 30	115	122		493		24.75
1970	Jan. 28	132	127	591	511	22.33	24.85

Preoperative, percutaneous kidney biopsy revealed normal renal tissue by both light and [illegible]lectron microscopy (Fig. 3). The capillary basement membranes [illegible]ere normal without any evidence of fusion of the [illegible]ncural epithelial foot processes, and no amyloid deposits were found. A percutaneous biopsy from the liver [illegible]as normal.

The postoperative course was uncomplicated. The serum albumin concentration became normal immediately after operation, four [illegible]eeks after surgery the proteinuria had vanished and the serum cholesterol was normal (Fig. 2). The patient was discharged 60 days postoperatively.

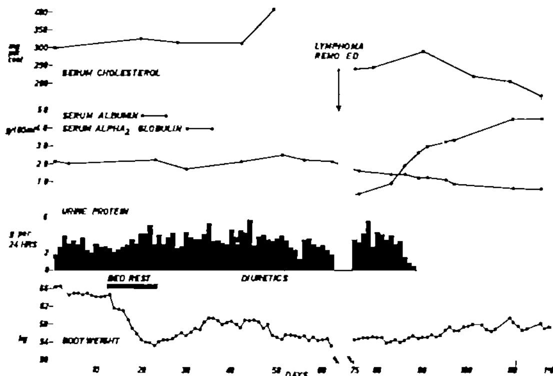


Fig. 2. The clinical course in case 1. Two months after admission tumor in the mediastinum was removed radically and dramatic change in the patient' condition was noted.

Two years after operation the kidney function is normal. Urinary sediment, serum electrophoresis and serum cholesterol are normal. There is no proteinuria and no edema. The patient' general condition is excellent and he is fully employed.

Case 2

A 17-year-old female who in Nov 1968 was admitted to local hospital with the nephrotic syndrome which had developed during six weeks. A chest X-ray revealed enlarged lymph nodes in both hilar regions and in the upper part of the mediastinum on the right side. During bed rest the edema disappeared and the patient was transferred to the Division of Radiotherapy in this hospital.

On admission on Jan. 2, 1969 her general condition was good; there was no edema, but enlarged lymph nodes in the right supraclavicular region were noted. Mediastinoscopy showed numerous small lymph nodes along the trachea and large lymph nodes in the mediastinum. Biopsy from these lymph nodes revealed Hodgkin disease of the mixed cellularity type.

Kidney function was normal (Table I) Proteinuria varied from 3.2 to 17.4 g/24 h, the serum albumin concentration was below 1 g/100 ml. The α_2-globulin concentration was increased to 0.9 g/100 ml and the γ-fraction was normal (Fig. 4). I urography revealed nothing abnormal and phlebogram demonstrated normal conditions of the inferior vena cava, and no compressions by lymph nodes were seen. The pressure gradient from the right atrium to point below the renal veins was 7.5 mmHg in the supine position. Lymphography revealed normal abdominal lymph nodes and the thoracic duct had normal configuration. A percutaneous kidney biopsy carried out on Jan. 22, 1969 showed minimal or no change on light microscopy (Fig. 5) No amyloid deposits were found.

On Jan. 24, 1969 cobalt treatment against lymph nodes in the axilla and mediastinum was started. This treatment continued until 3 700 rad had been given to two fields. Four days after initiation of therapy the proteinuria had disappeared and rapidly progressing normalization of the serum electrophoresis occurred.

On March 13, 1969 the patient was discharged. Kidney function has since been normal. No sign of recurrence of Hodgkin's disease has been seen. This patient is in good health 1 year after admission.

Case 3

A 53-year-old man who in Nov 1968 was admitted to the Division of Radiotherapy in this hospital. An axillary lymph node biopsy had shown Hodgkin disease of the mixed cellularity type: cobalt irradiation was given to lymph nodes in both axillary regions, in the neck and in the mediastinum. A total dose of 7 400 rad was given to four fields. The patient was without symptoms during the following year. The ESR was still elevated, but there was no proteinuria and kidney function was normal. One

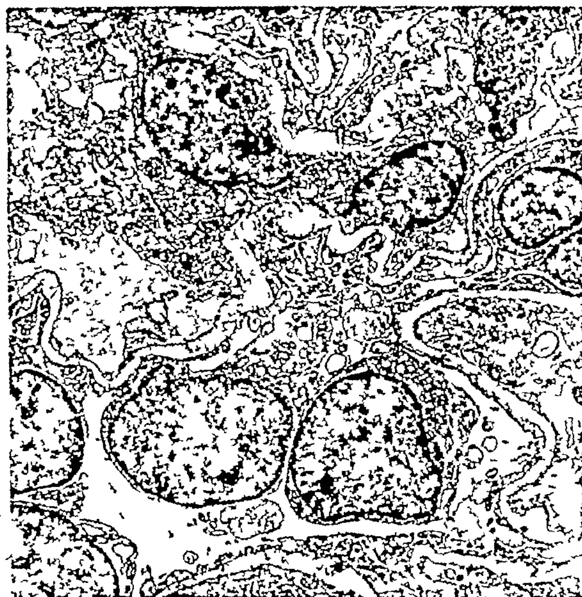


Fig. 5. Case 1. Electron micrograph from kidney biopsy showing normal structure of glomerular capillary wall. Glutaraldehyde-osmium fixation. × 9 730.

year after discharge this patient developed nephrotic syndrome and was readmitted to the Division of Radiotherapy. Cobalt irradiation was started against abdominal and inguinal fields and 3 700 rad were given to each field. As the nephrotic syndrome persisted with severe edema, the patient was transferred to Medical Department C.

On admission the patient's general condition was good; physical examination showed pitting edema of the lower extremities and edema of the abdominal wall. There was anemia with a Hb concentration of 10 g/100 ml. Serum creatinine varied between 1.1 and 1.5 mg/100 ml. Proteinuria ranged from 2.1 to 14.0 g/24 h, also in this patient a high serum cholesterol, low serum albumin and high serum α_2-globulin concentration were found. I.v. urography demonstrated normal kidneys and in the inferior caval vein a pressure gradient of 3 mmHg was measured in the supine position between the right atrium and a point below the renal veins. A lymphographic study carried out after cobalt treatment showed the retroperitoneal lymph nodes to be of normal size.

A percutaneous kidney biopsy was studied by light, electron and immunofluorescent microscopy. No significant change was noted by light microscopy. The only abnormality revealed by electron microscopy was a diffuse fusion of the visceral epithelial foot processes, the capillary basement membrane was normal (Fig. 6). Using an

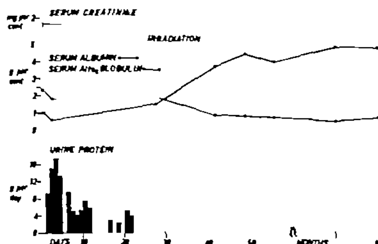


Fig. 4. The clinical course in case 2. Four days after initiation of cobalt treatment the proteinuria had disappeared. In the next four weeks serum proteins became normal too.

Fig. 5. Case 2. Kidney biopsy showing minimal or no glomerular changes. Sirius red staining, × 250.

immunofluorescence technique no binding of fibrinogen, complement or immunoglobulins could be demonstrated.

During diuretic treatment the edema disappeared, resulting in weight loss of 23 kg in the course of 21 days, but the other abnormalities indicative of the nephrotic syndrome were still present.

The nephrotic syndrome is still present in this patient; there is moderate anemia and kidney insufficiency has progressed, the serum creatinine now being 2.8 mg/100 ml.

DISCUSSION

Several authors have described a total of 23 cases of Hodgkin's disease accompanied by the neph-



Fig 6 Case 3 Electron micrograph from kidney biopsy showing normal structure of glomerular basement membrane and slight fusion of epithelial foot processes. Glutaraldehyde-osmium fixation, 19 300.

rotic syndrome In 12 of these patients percutaneous kidney biopsies have been carried out (4 5 11) In eight cases microscopy showed minimal or no changes in three cases amyloid deposits were demonstrated (5) and in two patients signs of venous obstruction were present (5). In the other ten patients no data concerning kidney pathology are presented In 1 cases the nephrotic syndrome disappeared during treatment of Hodgkin's disease (1 2, 7 9 10 11 13 15) and in six the nephrotic syndrome recurred at the same time as recurrence of the Hodgkin's disease (2, 7 10 11) A causal relationship between Hodgkin's disease and the nephrotic syndrome thus seems to be evident in patients without any signs of organic kidney lesions or venous obstruction

In our patients a classic nephrotic syndrome was present. In patients 1 and 2 edema and heavy proteinuria developed as initial events to the clinical appearance of Hodgkin's disease, whereas the nephrotic syndrome in patient 3 appeared later in the course of this disease.

The clinical and laboratory features of nephrotic

syndrome in these patients are quite similar to those in nephrotic syndrome associated with the minimal lesion, except for the presence of normal (cases 2 and 3) or high gamma globulin (case 1) In no case was paraproteinemia found. In all three patients the proteins excreted in the urine were albumin (35–100 %). Light as well as electron microscopy of the kidneys showed minimal or no change. No glomerular fluorescence was revealed by immunofluorescent microscopy

In patients 1 and 2 remission of the nephrotic syndrome was seen simultaneously with the treatment of Hodgkin's disease, regardless of whether by extirpation or cobalt irradiation. Remission took place during the course of a few days. Now 2 and 1 /₂ years have passed and no signs of recurrence of the disease are evident. Patient 3 still has an active Hodgkin's disease and the nephrotic syndrome persists unchanged.

The close time relationship as regards origin and remission of the nephrotic syndrome and Hodgkin's disease, as noted previously suggests a causal connection between these conditions. This is also indicated by the very rapid disappearence of all clinical, physiological and biochemical abnormalities of the nephrotic syndrome along with therapeutically induced remission of Hodgkin's disease in two of our patients.

The mechanism leading to the development of nephrotic syndrome in Hodgkin's disease remains unknown at the present time. It has been suggested recently that a barrier-held virus produces immune-complexes, which may evoke the Hodgkin's lesion (16). Such a concept might possibly serve to explain the coexistence of these two disease states.

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HEART RATE DURING EXERCISE IN PATIENTS WITH ATRIAL FIBRILLATION

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Abstract. A group of 195 patients with atrial fibrillation, mostly due to advanced valvular heart disease, have been examined by the graded work test and an orthostatic test when they were on maintenance digitalis therapy. The highest attained work load averaged 275 kpm/min, at an average heart (ventricular) rate of 134 beats/min. This corresponded to a load of 145 at heart rate 110. There was a wide range of work capacity. The individual relationships of heart rate increase to work load increase varied considerably from linearity but were approximately linear in many cases and the average for groups of patients were also relatively linear. Alinearity was observed especially in patients with very low work capacity. The increase in heart rate during an orthostatic test was 30 beats/min or more in 8% of the patients. A 'steady state' was obtained during the highest work load in 44% of the patients. Our experience is that, in atrial fibrillation, the graded work test must be evaluated with caution but often gives valid clinical information.

It is well known that, when a patient develops atrial fibrillation, his physical capability as a rule becomes reduced. Atrial fibrillation may be a severe complication in a cardiac patient in failure. In a patient with 'lone' atrial fibrillation, on the other hand, the reduction may often be slight and the patient may not even notice the cardiac arrhythmia.

One of the aims of the attempt to convert atrial fibrillation to sinus rhythm is to improve the patient's physical capability. The functional capacity and the work of the heart can be estimated more precisely by measuring hemodynamic data such as central pressures and cardiac output at rest and during exercise, less precisely by measuring heart rate and a few other clinical variables in an exercise test. In the literature it has been pointed out that there may be a steep and irregular increment in the heart (ventricular) rate during exercise in patients with atrial fibrillation (3, 16) but only a relatively small number of patients have been studied. The purpose of the present study is to describe the reaction in a standardized exercise test in a large group of patients with this arrhythmia.

MATERIAL

Exercise tests were made on 195 patients with atrial fibrillation. In patients who had performed more than one exercise test, only the first test was used in the present report. The repeated tests were used to study the reproducibility of the test reaction and the influence of different digitalis dosage on the exercise test, as will be reported separately. The composition of the patient group is shown in Table I. The group is dominated by patients with valvular heart disease, often referred to this hospital for consideration of cardiac surgery. The exercise test was part of this evaluation. A considerable number of the patients had already undergone cardiac surgery (in most cases mitral commissurotomy), and some of them were considered for further and more extensive cardiac surgery such as the insertion of valve prosthesis. Most patients thus had fairly advanced valvular disease, but they were in clinically stable condition at the time of the test and not in heart failure.

The subgroup 'congenital heart disease' consisted of four cases of atrial septal defect, of whom one also had mitral insufficiency, two cases of subvalvular aortic stenosis, of whom one also had mitral valve disease, and one case of ventricular septal defect combined with aortic insufficiency.

The subgroup 'miscellaneous' consisted of four cases of earlier myocarditis, three cases of arterial hypertension, three cases of unspecified valvular heart disease, two cases of coronary insufficiency, of whom one had had myocardial infarction, and two patients who had no diagnosed heart disease ('lone' atrial fibrillation).

All patients were on maintenance digitalis therapy (usually digoxin (Lanacrist®), but other types of digitalis drugs were also used. There were no clinical signs of toxic digitalis dosage in any of the patients.

Table I *Review of the case material with regard to underlying heart disease sex age and heart volume (usually measured in the sitting position, expressed per unit body surface area)*

Underlying heart disease	No of pats.	Sex		Age (y.)			Mean age (y.)	No. of pats. Heart volume (ml/m² BSA)			Mean heart volume
		♂	♀	< 40	40–50	> 50		< 550	550–750	> 750	
Mitral valve disease	98	37	61	22	46	30	47	25	40	33	720
Mitral and aortic valve disease	66	26	40	16	33	17	45	3	27	36	810
Aortic valve disease	3	3	—	—	1	2	51	—	1	2	850
VOC arterial hypertension	8	1	7	—	2	6	54	1	3	4	730
Congenital heart disease	7	2	5	1	1	5	51	—	3	4	660
Miscellaneous	13	9	4	1	8	4	48	6	4	3	620
Entire material	195	78	117	40	91	64	47	35	78	82	750

METHODS

The electrocardiogram was recorded with a direct writing four-channel apparatus (Mingograph 4, Elema Schönander Stockholm). The leads were usually I, II, III and V_1–V_6, sometimes V instead of V. The recordings were made at rest recumbent, during and after upright standing for 8 min (orthostatic test), and during as well as after exercise. During the exercise test the reference electrode for the precordial leads was placed on the forehead (CH leads) (7). The paper speed was usually 10 or 50 mm/sec, and the amplification was 1 mV = 10 mm.

The orthostatic test which preceded the exercise test was performed as more closely described by Sandberg (13). According to current nomenclature, the orthostatic test was considered 'positive' when the heart rate increased from the rest value by 20–29 beats/min, and 'pronounced positive' when the heart rate increased by 30 or more beats/min.

The exercise test was performed according to the graded steady state principle (14, 17) with an electrically braked bicycle ergometer (6). The patient cycled for 6 min at each of successively higher loads. The test started with a variable initial load (50–300 kpm/min), selected according to the presumed functional capacity of the patient. The ECG was continuously recorded during the test, and the heart rate was determined—as the average of 25 cardiac cycles—from the ECG tracings at the 2nd, 4th and 6th min on each load. The patients were clinically supervised, and the arterial BP was measured by the auscultatory cuff method. The test was continued according to the generally accepted rules, usually until the patient had either a heart rate level well above 170/min or dyspnoea and tachypnoea exceeding 35/min, angina pectoris, abnormal ECG changes, a fall in arterial BP or pronounced fatigue.

The result of the exercise test was expressed in different ways: as the work load at a heart rate of 110/min (W_{110}), as the highest work load performed for 6 min (W_{max}), as the increments in heart rate at different loads, and finally as the ability to obtain a steady state as defined below. W_{110} was calculated by slight interpolation or extrapolation, assuming a linear relationship between heart rate and work load within the small interval in question. A steady state was judged to be obtained when the difference between the heart rates at 2, 4 and 6 min on one load was 10 beats/min or less.

RESULTS

As expected, the working capacity of the patients was as a rule low. Thus the mean value of W_{110} was 145 kpm/min and of W_{max} 275 kpm/min (Table II). The range was wide in accordance with the heterogeneity of the patients studied. The highest obtained heart rate (at the end of the W_{max} load) averaged 134/min but varied considerably—when it was lower than 170/min some type of abnormal limitation or pronounced fatigue had appeared.

The orthostatic test was 'positive' or 'pronounced positive' in 23% of the patients (Table III). A steady state was obtained in 44% (Table IV).

The average increase in ventricular rate between three different loads is presented in Table V. The number of patients in this Table is reduced, since many were not able to continue on three different loads and since those cases were chosen in which the change in heart rate was measured at successive increases of the load by 100 kpm/min. (Some patients had other increases of load.) The heart rate increase from rest to the starting load was 37 beats/min in the total of 63 patients, and 32 in the 21 patients who had 100 kpm/min as starting load. The increase from the starting load to the second load was 14 and

Table II. *Result of the work test (In 16 cases the work test could not be evaluated in these terms)*

Underlying heart disease	No. of pats.	W_{130} Range	W_{130} Mean	W_{max} Range	W_{max} Mean	Final heart rate Range	Final heart rate Mean
Mitral valve disease	88	10–470	128	50–700	264	73–177	135
Mitral and aortic valve disease	60	20–530	161	50–800	275	77–171	133
Aortic valve disease	3	20–180	82	200–900	517	139–186	169
VOC+hypertension	8	30–320	169	100–400	238	87–172	121
Congenital heart disease	7	70–230	182	50–450	221	83–157	111
Miscellaneous	13	10–310	162	50–800	346	105–168	138
Entire material	179	10–530	145	50–900	275	73–186	134

16, respectively and the increase to the third load 19 and 19 respectively. Thus there is a more pronounced increase from the resting value of heart rate to the value at the first load than between the other loads. The same tendency is shown in Fig. 1 where individual values have been plotted for the 19 patients (not included in Table V) whose successive increases of load were 200 kpm/min. It should be remembered that the increase in oxygen uptake from rest to a low work load is larger than the same increase at a high exercise intensity. In Figs. 2–4 the results have been plotted according to the W_{max} value in patients who performed two or more different work loads and had 130 or more in final heart rate. The Figures illustrate that patients with a higher W_{max} have a more linear relationship between work load and heart rate than patients with a low W_{max}.

DISCUSSION

The case material predominantly consisted of patients with valvular disorders and of a relatively low age and is therefore not representative of a general clinical material of patients with atrial fibrillation (1).

The orthostatic test in our patient group showed a heart rate increase which was 'positive' or 'pronounced positive' in altogether 23% of the cases. This is a higher proportion than in normal subjects of the same age. The patient group was divided according to heart rate increase at the orthostatic test. The half with a larger increase had an average W_{130} of 72 kpm/min and W_{max} of 237 kpm/min, as compared to the values of 128 and 271 in the other half.

In a discussion of the response to exercise tests in patients with atrial fibrillation it is necessary first to consider the nature of the ventricular response in this arrhythmia (18). In the past the irregularity of the ventricular rate has been regarded as completely haphazard. Some authors,

Table III. *Result of the orthostatic test (not performed in three cases)*

Heart rate 20–29 beats/min was considered as positive test and more than 29 beats/min pronounced positive

Underlying heart disease	Total no. of pats.	No. of positive tests	No. of pronounced positive tests
Mitral valve disease	97	17	7
Mitral and aortic valve disease	64	6	3
Aortic valve disease	3	—	1
VOC+hypertension	8	1	1
Congenital heart disease	7	1	1
Miscellaneous	13	3	3
Entire material	192	28	16

Table IV. *Result of the work test with regard to achieved steady state on highest load. (19 cases the steady state could not be evaluated)*

Underlying heart disease	No. of pats. Steady state achieved	Steady state not achieved	Total
Mitral valve disease	40	51	91
Mitral and aortic valve disease	25	39	64
Aortic valve disease	2	1	3
VOC+hypertension	5	3	8
Congenital heart disease	5	2	7
Miscellaneous	5	8	13
Entire material	82	104	186

Table V. Heart rate at rest and at three different loads with a successive increase of 100 kpm/min, measured in 63 patients who performed the work test with three loads. Load I is the starting load

Starting load (kpm/min)		Heart rate							
		Rest		Load I		Load II		Load III	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
50	13	76	57–96	111	85–144	119	101–164	141	111–171
100	21	69	44–98	101	69–146	117	77–155	136	96–168
150	8	76	57–102	116	86–154	131	111–161	149	123–170
200	9	72	58–88	108	93–128	1 4	111–139	141	126–149
250	8	71	56–90	115	94–133	128	116–139	151	139–169
300		64	—	115	—	130	—	147	—
Total	61	72	44–102	108	69–154	121	77–164	141	96–171

however have shown that this is not always true (2, 10, 15). Söderström (15) studied the R R intervals in atrial fibrillation and found a tendency to grouping of the intervals at certain 'levels' e.g. time-lengths. Atrial fibrillation differed from atrial flutter in that the dominant R R intervals did not seem to be multiples of the atrial period (F F interval). Therefore Söderström postulated that the R R intervals were multiples of the refractory period of the A V junction. Drugs as well as changes in autonomic nervous tone would affect the ventricular response in atrial fibrillation by changing the A V conduction time or the A V refractory period.

Horan and Kistler (8) calculated the variation in ventricular response in 47 ECG tracings on 17 patients with atrial fibrillation. The distribution curve of the R R intervals on these recordings also revealed some type of pattern as below.

1) At rates more than 140 beats/min the ventricular intervals appeared to be clustered about a single narrow peak of the distribution curve, most R R intervals being of the same time-length.

2) At intermediate rates there were two peaks (around 90 and 120/min), i.e. two different time lengths.

3) At rates lower than 90 beats/min there was a main peak, corresponding to the longest R R interval of those mentioned in 2), and an even distribution along a wide range of time-lengths.

The two discussed studies are convincing in their conclusion that the ventricular response in atrial fibrillation is not completely haphazard.

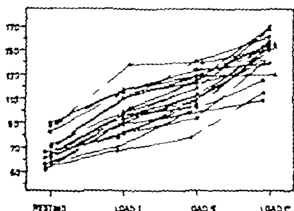


Fig. 1. Heart rate at rest and at three different loads with a gradual increase of 200 kpm/min. ● = starting load of 50 kpm/min, ○ = 100, ▲ = 150 and Δ = 200 kpm/min.

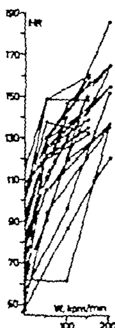


Fig. 2. Patients with W_{max} of 0–49 kpm/min.

However the reason for the grouping of the R-R *intervals in this arrhythmia is not known. The* exact mechanism of the A V conduction is as yet poorly defined in atrial fibrillation. It has been suggested that the grouping of R R intervals *is the result of either a partial A V junctional* penetration (a type of concealed conduction) or a dual A V junction pathway (9 11) Another factor of possible importance in cases with a very rapid ventricular rate is that the R R intervals could also be related to the effective refractory period of the ventricle. The mechanism behind the dual grouping may contribute to the lack of linear relationship between work load and heart rate in cases of atrial fibrillation.

In patients with atrial fibrillation it is often stated that exercise accelerates the ventricular response much more than the same exercise would do when the patient had been restored to sinus rhythm. However when the patient is adequately digitalized there is usually no excessive increase in the heart rate following exercise (12) With regard to the mechanism previously suggested it is interesting that both vagal stimulation and digitalization seem to increase the possibility of concealed conduction (4). The slow ventricular *rates in a digitalized* patient with atrial fibrillation could be explained by an increased number of concealed impulses. Thus the ventricular response during exercise could depend upon a decreased number of concealed impulses, for example accentuated sympathetic tone or an altera*tion of the A V junction refractory period.*

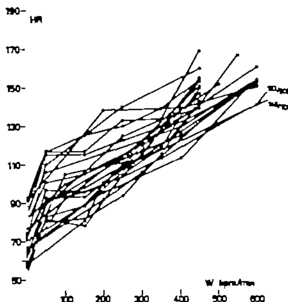


Fig 4 Patients with W_{max} of more than 449 kpm min.

Another factor that might be of importance with regard to the ventricular response is the fibrillatory activity in the atria. The rates of the fibrillatory waves at different exercise loads are not known. However there is no reason to believe that the change of the bombardment of the A V junction by fibrillatory impulses could play any important role. The fibrillatory activity is of such a rate that there is always an impulse on hand to penetrate the A V junction. Therefore the decisive factor for the change of the ventricular rate at different loads is whether the decrease of the refractory period is gradual (i e has a linear relationship to the increase of load) or not.



Fig. 3 Patients with W_{max} of 250–449 kpm/min.

Only few reports, with small case materials, have been published on the exercise performance *in patients with atrial fibrillation (3 5 16).* A general opinion has been that the ventricular response is irregular to such a degree that the individually diagnostic or prognostic value of exercise tests in patients having the arrhythmia is doubtful. However in our series of digitalized patients there is a certain regularity in the average increase of the ventricular rate at different loads,

as seen in Table V and Figs. 1–4 In Fig. 1 the rate increase at different loads is relatively uniform with one exception. As seen from Figs. 2–4 there is a more linear relationship between work load and heart rate in patients with a higher work capacity Further another parameter for the evaluation of the exercise tests, namely attainment or non-attainment of 'steady state' should be considered. Almost half of the patients attained a 'steady state' which is higher than we expected considering the composition of the material with a low functional capacity and degree of training.

In conclusion, the ventricular response to exercise in patients with atrial fibrillation is often irregular in relation to work load, but in the majority of cases a certain pattern is seen. In exercise at different loads there is often a uniform change of the ventricular rate related to the change in load.

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FATTY ACID COMPOSITION OF ADIPOSE TISSUE IN MALE NORWEGIANS WITH MYOCARDIAL INFARCTION

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Abstract. Gas-chromatographic studies of the fatty acid composition of adipose tissue specimens have been carried out in 70 urban men with acute myocardial infarction (AMI) and in control group comprising 25 healthy men of comparable age. Only subjects living free of dietary instructions ere included in the study Samples of adipose tissue obtained from patients with infarction ho had had known coronary heart disease (CHD) for at least one year contained significantly higher mean linoleic acid than samples from patients with no history of previous heart disease or from healthy subjects. The difference between mean linoleic acid content in the two latter groups was also statistically significant, with the lowest values found in patients with infarction but without known previous CHD

Diet is assumed to be the dominant factor responsible for the discrepancy in fatty acid composition. Patients with known CHD have probably been more assiduous in following recent widespread nutritional advice recommending increased intake of polyunsaturated fats than the population as whole.

Male Norwegians living in urban areas exhibited almost the same adipose tissue fatty acid patterns as the inhabitants of countries with high incidence of CHD.

Dietary changes have been recommended for the prevention of atherosclerosis and coronary heart disease (1, 12). The recommendations include reduced intakes of saturated fats, cholesterol and simple sugar and an increased intake of poly unsaturated fats. These recommendations have been supported by prospectiv clinical trials showing favourable effects (5 14 15 18). Dietary recommendations have been directed particularly towards groups with clinical atherosclerosis, but have also been given to the whole population (12). Whether general recommendations without dietary controls are effective in changing dietary habits has not been systematically studied.

According to, among others, Hirsch et al. (6) studies of the fatty acid composition of human depot fat represent a valuable help in estimating the results of changes in the composon of dietary fats. In the present investigation the fatty acid composition of adipose tissue lipids has been studied in two groups of men admitted to hospital with AMI, one group with a history of previous CHD and the other comprising men who had not experienced symptoms of heart disease prior to the onset of the infarction. The purpose of the study was to ascertain whether previous CHD had resulted in an increased impetus to follow the general dietary recommendations.

A control material of men without myocardial infarction was included in the study

MATERIAL AND METHODS

The material consisted of total of 79 men with AMI admitted to the coronary care unit in 1970 43 subjects without previous heart disease, and 27 with history of CHD (AMI and/or angina pectoris) of at least 12 months duration. The age group selected was 40 to 70 years, and in both groups the mean age was 58–59. Twenty-five men in the same age group who had been admitted to the surgical ward for minor ailments (varices, fractures, hernia) acted as controls.

Prior to hospitalization all subjects had been following their usual diets free of control and with no specific dietary instructions. None had diabetes mellitus or other diseases known to affect lipid metabolism.

Biopsy specimens of subcutaneous adipose tissue from the buttocks were obtained within the first 3–5 days after admission. For analysis the samples were transected and extraction of total lipids was performed with 1 1 mixture of acetone and absolute ethyl alcohol. After cleaning procedure with petroleum ether 40–60% approximately 10 mg samples were hydrolysed and methylated according to the method of Stoffel et al. (17). Gas-chromatography was carried out on Perkin-Elmer

Table I *Proportions of major fatty acids in adipose tissue*

Means with S D in parentheses

	Myocardial infarction			Statistical evaluation		
Fatty acids,	A. Without previous CHD (N = 43)	B. With previous CHD (N = 27)	C. Controls (N = 25)	A B	A–C	B–C
Myristic	3.8 (1 1)	3 1 (0.6)	3.5 (1.2)	$p<0.01$	n.s.	n.s.
Palmitic	20.2 (1 7)	18 8 (1.8)	20 8 (2.3)	$p<0\ 01$	n.s.	$p<0.01$
Palmitoleic	8 6 (1 6)	7 7 (1.6)	7.3 (3.3)	n.s.	$p<0\ 05$	s.
Stearic	4 2 (1.3)	4 1 (0.8)	5.3 (1.5)	n.s.	$p<0\ 01$	$p<0\ 01$
Oleic	47 0 (2.5)	46.3 (2 0)	45.5 (3.5)	s.	$p<0\ 05$	n.s.
Linoleic	8 0 (2.2)	11.5 (2.4)	9.5 (2.9)	$p<0\ 001$	$p<0.05$	$p<0\ 05$
Eicosenic	2.6 (0 6)	2 6 (0 9)	3.2 (0.9)	n.s.	n s.	s.

F 11 ppartus with flameionization detector at column temperature of 195 The stationary phase of the column was chromosorb W 60–80 mesh, and the liquid phase 8% butandiol succinate polyester The methods and procedures have been described in detail elsewhere (13). Calculations of the fatty acid composition from the gas-chromatographic patterns were made by multiplication of relative retention time by peak height, both measured in mm on the recording paper (2). The products of all fatty acids were added and the percentages calculated for the individual fatty acids.

Statistical evaluation of differences between means was done by means of Student's *t*-test (16) *p*-values higher than 0 05 were not considered to be significant.

RESULTS

Table I shows the results of the gas-chromatographic analysis, mean values, standard deviation (S D) and the results of the statistical evaluation of differences between means are recorded. Percentages of fatty acids below 1 were regarded as trace amounts and are not included in the table.

The percentage of linoleic acid was significantly higher in the group of men with previous CHD than in the two other groups: 11.5% compared to 8 0% in the group of patients with infarction but without previous CHD and 9.5% in the controls. The relatively small difference between the means in the two latter groups was also significant.

An increase in palmitoleic and oleic acid and a decrease in stearic acid were found in the group with myocardial infarction without previous CHD when compared with healthy subjects. Although other minor differences were noted, they were usually not significant.

DISCUSSION

Studies of the fatty acid composition of tissue lipids have demonstrated the dependence upon the composition of dietary fat. Of the major fatty acids occurring in human depot fat, linoleic acid is not synthesized by the body but is totally derived from the intake of linoleic acid in the diet. Palmitic, palmitoleic, stearic, and oleic acids may either originate from the diet or be synthesized endogenously The percentage of linoleic acid therefore reflects the composition of the diet with regard to this fatty acid. In man it has been shown that diets rich in linoleic acid increase the proportion of the linoleic acid in depot fat. The increase occurs over a long period, and one report demonstrated a steady increase in linoleic acid percentages over the entire period of observation (3–4 years) on a cholesterol-lowering diet rich in polyunsaturated fatty acids (3).

In the present study values for linoleic acid in depot fat were higher in men with a previous history of CHD than in men who had not suffered earlier heart disease. The difference indicates a dissimilarity in the composition of dietary fat, and possibly a higher intake of vegetable oils, rich in linoleic acid, in men with known CHD Although any interpretation of the magnitude of the changes in the adipose tissue fatty acids in terms of diet composition is difficult, it is probable that at least some of the patients with previous CHD had followed more closely the diet recommendations. The low levels of linoleic acid in the controls and in patients without previous CHD demonstrate that the advice given in diet campaigns did not apparently affect these groups to

any noticeable extent. This finding contrasts with observations in the USA where in the years 1962–1966 an increase in linoleic acid percentages was demonstrated and taken as an indication of changes in the composition of the diet of the whole population (10).

Our investigation of patients with AMI leaves open the question whether or not the alterations in fat depot reported here are associated with a favourable dietary influence on the morbidity of CHD. It is possible that the dietary changes were inadequate. In men adhering strongly to diets high in polyunsaturated fatty acids, Christakis et al. (3) and Dayton et al. (4) found that the mean values of linoleic acid content of the subcutaneous fat increased to 18.9 % and 24 % respectively which was approximately twice the values for subjects subsisting free of dietary control. It is therefore apparent that the differences between the groups reported in the present study are relatively small compared with the larger changes usually seen in controlled dietary studies.

The difference concerning linoleic acid percentages reported here between the group of men with myocardial infarction but without previous CHD and the control material is small, but statistically significant. This finding indicates a possible difference in dietary habits between the groups. To confirm this conclusion with certainty however would probably require more extensive studies, and the possibility of differences in metabolism cannot be ignored.

It is difficult to compare the present results with similar investigations from other countries. In addition to complex dietary habits, the fatty acid composition of depot fat is influenced to some extent by race and sex (7). In spite of these potential sources of errors, it may be stated that male Norwegians living in urban areas exhibit almost the same depot fat composition as found in the general population in other western countries with high incidence of CHD (9). On the other hand, the composition of depot fat in male Norwegians seems to differ from values in urban men residing in areas where the risk of arteriosclerotic heart disease is much lower. In Japan CHD occurs with rates of incidence and mortality 25 % less than the rates of western countries (11). Japanese composition of depot fat shows higher average linoleic acid (16.5 %), lower oleic acid (43.0 %) and lower stearic acid (3.6 %) (9). The discrepancies probably reflect differences in levels and kinds of dietary fats, with a predominance towards vegetable fats in the Japanese (8).

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CENTRAL HEMODYNAMICS IN SEVERE POISONING BY HYPNOTIC DRUGS

Coma Depth and Body Temperature

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Abstract. Hemodynamic data have been recorded in nine patients during initial and late phases of severe poisoning by hypnotic drugs. Cardiac output (C.O.) was determined by the dye dilution method and right heart catheterization using "floating" catheter technique. Oxygen uptake was calculated from C.O. and arterio-venous oxygen difference. During deep coma, characterized by hypothermia, the C.O. stroke volume and oxygen uptake were significantly decreased and the peripheral vascular resistance markedly increased. The arterio-venous oxygen difference was generally normal, which implies that C.O. varied with oxygen uptake. Central venous pressures were normal or somewhat low as also were the pressures in the pulmonary artery. During recovery the heart rate, stroke volume and C.O. increased in proportion to the increase in tissue metabolism as evaluated from the calculated oxygen uptake. The reduced tissue metabolism, reflected in reduced body temperature and low oxygen uptake, seems to be the main explanation of the hypo-kinetic circulation in patients with deep coma because of poisoning by hypnotic drugs. The rather frequent roentgenological signs of pulmonary congestion or edema during the course of intoxication with hypnotic drugs do not seem to be explained by increased left ventricular filling pressures as reflected in elevated pulmonary artery diastolic pressures. A toxic effect on the vascular bed with increased permeability may be the most important underlying explanation.

The mortality rate of patients with severe poisoning by hypnotic drugs was earlier high and death occurred in a picture of clinical shock. Following the introduction in Scandinavia of modern intensive care, based on support of vital functions, the mortality rate decreased markedly (8, 16, 4) The hemodynamic pattern in severely ill patients with or without a picture of clinical shock may vary and the value of hemodynamic measurements in these cases is well established (10 18 27) Many of these cases in severe shock still present difficult diagnostic and therapeutic problems. Disagreement exists concerning the proper treatment of patients with severe poisoning by hypnotic drugs complicated by arterial hypotension and shock. Furthermore, cases with severe poisoning may be complicated by a clinical picture of pulmonary edema at various stages during the course of the poisoning (20, 26, 28) Repeated controls by pulmonary X-ray may reveal signs of pulmonary stasis or edema even in cases without clinical signs of pulmonary congestion. Especially in young patients it has been observed that pulmonary edema may develop into a fulminant form which in some cases is resistant even to respirator treatment.

In the light of these observations, and since earlier reports in this field are scanty and to some extent also controversial, we have considered it to be of theoretical and practical importance to further analyse the central hemodynamics of patients with severe poisoning by hypnotic drugs at various stages of the disease as evaluated from coma depth and body temperature.

MATERIAL

The material consisted of 10 patients, 3 men and 7 women. The mean age was 33 years (range 21 71) Values for age, body weight and height are presented in Table I. In cases 1 9 two or three hemodynamic measurements were performed at various stages during the recovery from the poisoning. In case 10 a single investigation was performed during a later phase of the recovery from the poisoning when clinical signs of pulmonary edema were present. This case is represented by single dots in the figures but

Table I *Some anthropometric data for 10 patients with drug poisoning*

Case no.	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m^2)	Type of intoxication	Duration of coma (h)	Temperature (°C) Min.	Temperature (°C) Max.	Respirator treatment
1		2.	167	52	1.58	Butenemal (Diminal) + nitrazepam (Mogadon)	36	33.5	38.3	+
2	♀	46	170	62	1.73	Glutethimid (Doriden)	54	33.1	38.4	+
3	♂	21	176	70	1.87	Chlorpromazine (Hibernal)	72	31.0	37.8	+
4	♀	22	171	52	1.62	Butenemal-allypropymal (Diminal duplex)	60	26.8	38.9	+
5		24	161	62	1.64	Dibenzepin (Neodalit) + nortriptylin + butenemal-allypropymal (Diminal duplex)	60	30.2	35.2	+
6	♂	71	169	75	1.85	Butenemal-allypropymal (Diminal duplex) + alcohol	72	31.1	38.5	+
7	♂	79	160	50	1.51	Amitriptylin (Triptyl) + chlorprothixen (Truxal)	60	32.4	37.2	+
8	♀	.5	159	48	1.47	Propiomazine (Propavan)	24	31.2	37.9	–
9	♀	.3	165	65	1.71	Mebumal (Nembutal)	60	30.3	39.2	+
10	♀	46	158	54	1.54	Meballymalnatr.-allyl-bromallylbarb.-hydroxy-zine (Vesparax) + amitriptylin (Tryptizol)	36	34.1	39.2	+

has not been included in the statistical calculations of the hemodynamic data presented in Table II.

All patients included in the study presented a picture of severe poisoning following ingestion of hypnotic drugs. The duration of coma averaged 53 hours (range 24–72). The lowest measured body temperature averaged 31.4°C (range 26.8–34.1) and the highest 38.1°C (range 35.[illegible]–39.[illegible]). At the time of the initial hemodynamic study during deep coma the body temperature averaged 32.9°C (range 30.3–34.4). Duration of coma, body temperature and type of poisoning are presented in Table I. In five cases coma was caused by one drug: butenemal-allypropymal (Diminal duplex®), mebumal (Nembutal), glutethimid (Doriden®), chlorpromazine (Hibernal®) and propiomazine (Propavan). In the five remaining cases the poisoning was due to a combination of drugs.

Immediately after arrival at the hospital the patients were transferred to the Medical Intensive Care Unit. All studies were performed at the bedside, and an attempt was made not to interfere with the supportive therapy and general care of the patients.

All patients but one were given a period of respirator treatment. During 10 of the 21 hemodynamic measurements positive pressure ventilation (Engström respirator) was used (Table II). Oxygen was added if there was evidence of hypoxemia. During the coma electrolyte solution, on an average 3.7 l/day was given parenterally. This resulted in adequate urinary flow. In two cases (nos. 1 and 6) a small amount of norepinephrine was added to the infusion initially because of marked hypotension. During the initial study the patients were deeply comatous. During the final study they were in a comparatively light coma and two patients were even fully conscious. The total time of care at the intensive care unit averaged 4.7 days.

METHODS

Each of the 21 investigations included measurements of C.O. arterial and central venous blood pressure, heart rate, blood gases and rectal temperature. On 18 occasions catheterization of the pulmonary artery was performed, with determination of pressure and arterio-venous oxygen difference over the pulmonary vascular bed. A polyethylene catheter (PE 60) connected to a pressure transducer was advanced into the pulmonary artery via an outer teflon catheter inserted into either of the subclavian veins. The position of the polyethylene catheter was determined by observation of the pressure curve (2). A teflon catheter was also inserted into the femoral artery. Pressures were recorded on a direct-writing ultraviolet multichannel recorder (ABEM Ultralette). Mean pressures were obtained electronically. All patients were supine throughout the experiment. The mid-thoracic level at the insertion of the fourth rib at the sternum was taken as reference for zero pressure. C.O. was measured by the indicator dilution method, using indocyanine green (1.1 ml of a 0.50% solution). The dye was injected as a bolus into the pulmonary artery or in three cases, a subclavian vein. Blood was drawn from the femoral artery through a Beckman Cardio-Densitometer at a flow rate of about 20 ml/min, using a 50 ml glass syringe adapted to a Harvard pump. Dye curves were analysed according to the conventional Hamilton technique (12). Stroke volume and peripheral vascular resistance were also calculated.

Arterial and mixed venous blood was sampled simultaneously and the O_2-content was determined spectrophotometrically (Beckman B spectrophotometer). Arterial pH and P_{CO_2} were measured either by electrode or Astrup technique.

Table II. *Hemodynamic data of 10 patients with drug poisoning*

AVD = arterio-venous oxygen difference, CVP = central venous pressure, FA = femoral artery, PA = pulmonary artery, BE = base excess, R_s = systemic vascular resistance, S = systolic, D = diastolic, M = mean

Case no.	Body temp. (°C)	Heart rate (beats/min)	C.O. (l/min)	AVD (ml/l)	Oxygen uptake (ml/min STPD)	Stroke volume (ml)	Pressures (mmHg) CVP	FA S	FA D	FA M	PA S	PA D	PA M	R_s (U)	Pa_{O_2} (mmHg)	Pa_{CO_2} (mmHg)	BE (mEq/l)	pH	Respirator treatment
1	34.0	50	2.7			54	6	115	90	99				36.6	169	31	−4	7.41	−
	34.7	86	3.7			43	10	111	55	73	17	5	9	19.7					+
	36.8	79	4.3	37	157	55	7	95	42	56	12	4	8	13.0	105	35	−3	7.40	−
2	34.2	70	3.0	35	106	43	6	132	70	95	24	11	15	31.7	90	35	−7	7.33	+
	38.2	98	6.3	31	197	64	0	125	67	88	16	3	7	13.9	68	36	−1	7.41	−
3	31.0	79	4.0	38	152	51	2	125	74	93	14	8	10	23.3	91	32	−4	7.40	−
	37.2	86	8.5	50	421	99	4	138	76	100	23	9	16	11.8	92	34	+2	7.48	−
4	34.4	98	3.9	45	174	40	4	80	60	67				17.2	93	22	−2	7.49	+
	37.8	100	7.0	43	298	70	3	117	68	84	33	21	25	12.0	84	33	−4	7.38	−
5	30.3	68	2.9			43	3	70	50	57				19.7	28	38	−7	7.33	−
	32.5	76	4.1	49	167	54	0	112	71	87	15	8	10	21.2	109	24	−4	7.46	+
6	32.8	59	4.4	38	167	73		90	39	56	19	7	13	12.7	105	43	+3	7.42	+
	37.0	86	7.9	32	255	91	3	131	51	78	22	6	12	9.9	52	33	±0	7.45	−
7	31.1	79	5.6	28	133	71	3	129	81	100	22	15	17	17.9	140	26	−2	7.48	+
	36.0	108	10.5	21	221	97	3	117	66	83	31	16	24	7.9	190	54	−2	7.29	−
8	33.9	75	2.3	61	152	32	−1	97	60	75	15	7	9	30.0	69	47	+4	7.40	−
	37.4	113	3.3			47	7	113	62	78	20	9	13	14.7	72	39	±0	7.41	−
9	32.1	64	4.0	64	238	61	7	112	72	88	27	14	19	22.0	42	25	+2	7.56	+
	36.3	110	7.8			70	5	100	56	70	26	17	20	9.0	52	29	−2	7.46	+
	39.2	125	9.9	47	464	79	8	94	58	70	33	22	23	7.1	66	37	+5	7.49	+
10	37.7	128	5.1	48	43	40	4	100	60	74	38	20	25	14.5	106	35	+8	7.56	+

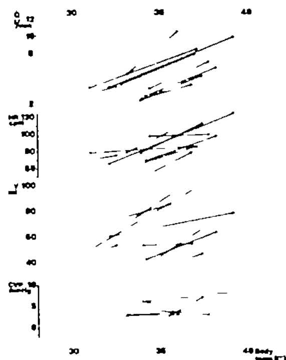


Fig. 1. Cardiac output (Q), heart rate (HR), stroke volume (SV) and central venous pressure (CVP) in relation to body temperature (°C), used as a measure of coma depth in 10 patients with severe poisoning by hypnotic drugs.

RESULTS

The results obtained during the hemodynamic measurements are presented in Table II. The data d in the figures are from the initial meas- when the patients were in deep coma, ..d from the final measurements when they were in light coma or almost awake. In two cases the results of a third investigation ha e been plotted.

Hemoglobin concentration, acid-base balance and arterial blood gases. The Hb concentration averaged 14.6 g 100 ml blood (range 11.7–18.0). Arterial P_{O_2} was markedly reduced in cases 5, 6 and 9 in spite of oxygen being added to the inspired air. This implies considerable physiological shunting of blood in the lungs. On seven occa sions oxygen tensions were increased above normal because of additional oxygen supply. Most cases hyperventilated somewhat or were slightly over ventilated, which in some instances resulted in a mild respiratory alkalosis. In case 7 there was a moderate alveolar hypoventilation with respiratory acidosis during the second study. Two cases (nos. 2 and 5) presented a slight metabolic acidosis initially.

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Heart rate increased from an average of 72 beats/min (range 50–98) during the first determination to an average of 97 beats/min (range 76–125) during the last study. This increase, on the average 25 beats/min (range –59), was significant ($p < 0.01$).

Stroke volume increased from an average of 52 ml (range 32–75) at the initial determination

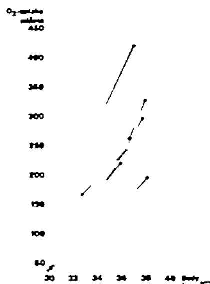


Fig. 2. Oxygen uptake (VO_2) (ml STPD min) in relation to body temperature (°C) in seven patients, comatous from hypnotic drug poisoning.

to 73 ml (range 54–99) at the final measurement. The increase, on the average 22 ml (range 1–48) was significant ($p < 0.01$)

Oxygen uptake in six cases was calculated from the arterio-venous oxygen difference and C.O. In Fig. 2 the oxygen uptake is shown as a function of body temperature. Within the range of the present observations oxygen uptake increased by 27 ml/ C increase in body temperature according to the regression equation, oxygen uptake (ml/min) = 27 × (body temperature) − 722, where S.D (y) = 85 and the correlation coefficient = 0.67

Intravascular pressures. The mean pressure in the femoral artery was on the average 81 mmHg (range 56–100) during the initial study and 80 mmHg (range 56–100) during the final determination. The systolic arterial blood pressure increased from an average of 106 mmHg (range 70–132) during the initial study to 116 mmHg (range 94–138) during the late stage of poisoning. The diastolic arterial blood pressure was relatively unchanged from an average of 66 to 62 mmHg. The pressures in the pulmonary artery were within normal limits in most cases. In cases 4 9 and 10 the pressures were slightly elevated during the final investigation (Fig. 3). There was no significant change in mean pulmonary arterial pressure between the initial study average 14 mmHg (range 9–19), and the final study average 16 mmHg

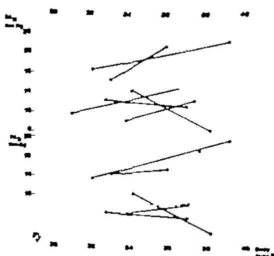


Fig. 3. Pulmonary arterial mean pressure (PA_m) and pulmonary arterial diastolic pressure (PA_d) in relation to body temperature as measure of drug-induced coma depth.

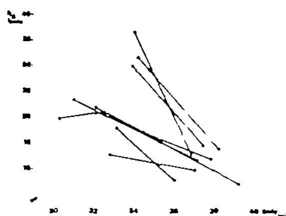


Fig. 4. Peripheral vascular resistance (U) in relation to body temperature as measure of coma depth in 10 patients with drug-induced coma.

(range 7–25). The systolic pressure in the pulmonary artery was on the average 20 mmHg (range 14–27) during the initial and 23 mmHg (range 12–33) during the final determination. The diastolic pressure was also normal and relatively unchanged between the two investigations. Central venous pressure was normal or somewhat low and showed no significant change during the course of the intoxication. No correlation between changes in central venous pressure and C.O could be demonstrated. In six of the cases there were roentgenological signs of pulmonary congestion or edema but there was no correlation to the pressures in the pulmonary artery

Systemic vascular resistance was expressed as the ratio between mean arterial blood pressure and C.O disregarding the small differences in right atrial pressure. In the stage of deep coma the peripheral vascular resistance was significantly elevated ($p < 0.001$) when compared to values for healthy subjects of corresponding age (1). During the recovery from the poisoning the peripheral vascular resistance decreased significantly ($p < 0.001$) (Fig. 4).

DISCUSSION

The present study of the hemodynamic changes during the course of severe poisoning by hypnotic drugs was made mainly on young patients and in no case was there any known underlying cardio-

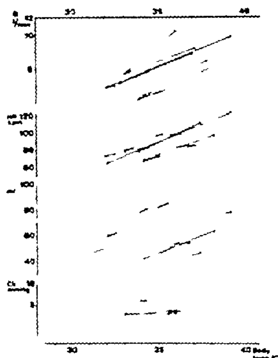


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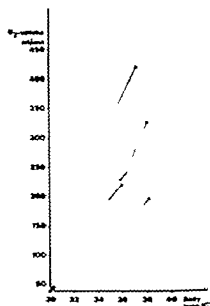


Fig. 2. Oxygen uptake ($\dot{V}O_2$) (ml STPD/min) in relation to body temperature (°C) in seven patients, comatous from hypnotic drug poisoning.

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HYPOTENSIVE EFFECT OF METHYLDOPA IN RENAL FAILURE ASSOCIATED WITH HYPERTENSION

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Abstract. Increased sensitivity to the hypotensive action of methyldopa is often seen in patients with renal failure. It is generally accepted that the increased sensitivity is due to drug accumulation in renal failure, though there are very few studies on this topic. The hypotensive effect and the serum concentration of unconjugated methyldopa have been determined in 6 patients with advanced renal failure and 7 patients with essential hypertension and normal or slightly reduced renal function. Following i.v. injection of 200 mg methyldopa the maximal decrease of the supine mean BP was on an average 21.5% in patients with renal failure and 11.5% in patients with essential hypertension without renal insufficiency. The maximal response was somewhat delayed in renal failure. The supine, standing and post-exercise BP was recorded during short-term oral treatment in the two groups of patients. The average reduction of the mean BP in patients with renal failure was 11, 16 and 20% respectively. In patients with essential hypertension the figures were 16, 26 and 25% respectively. The slightly higher hypotensive effect in the patients with normal renal function, however, was achieved after an average serum concentration of unconjugated methyldopa amounting to more than 4 times that found in the patients with renal failure. The study demonstrated an augmented sensitivity to the hypotensive effect of methyldopa in renal failure. The high sensitivity to the hypotensive action of the drug was not due to increased serum concentration of unconjugated methyldopa.

Although methyldopa was introduced in clinical medicine as an antihypertensive drug more than a decade ago, some of its effects are still poorly understood (5, 6, 13). The drug has been widely adopted in the treatment of hypertension associated with renal failure (7) because renal plasma flow and glomerular filtration are not reduced during therapy (1, 8, 16). Even an increase in the renal fraction of cardiac output has been reported (14).

However, many patients with renal failure are very sensitive to the hypotensive effect of methyldopa (3, 4). This increased sensitivity is of great clinical importance, but rather few studies have been performed concerning the hypotensive action of methyldopa in azotemic patients as compared with essential hypertension with normal or only slightly impaired renal function. As the drug is generally assumed to be eliminated by renal excretion, a high sensitivity in patients with renal failure might simply be explained by drug accumulation and high serum or tissue concentrations (2, 4, 9, 15). As far as we know, no experimental data on this topic exist.

The aim of the present work was to study the hypotensive effect of methyldopa after i.v. and short-term oral administration in relation to serum concentrations achieved in patients with advanced renal failure associated with hypertension and in essential hypertension without renal insufficiency.

MATERIAL AND METHODS

Thirteen patients with arterial hypertension were studied during stay in hospital. Table I gives some pertinent data of the patients. Group I consisted of 6 patients with advanced renal disease associated with hypertension. Their average blood urea concentration was 172 mg/100 ml, serum creatinine 11.8 mg/100 ml, and endogenous creatinine clearance 8.5 ml/min. The pre-treatment mean BP in the supine position in this group was on an average 141 mmHg. Group II consisted of 7 patients with essential hypertension and a normal or only slightly reduced renal function. The average blood urea concentration in this group was 46 mg/100 ml, serum creatinine 1.3 mg/100 ml and creatinine clearance 87 ml/min. Their supine mean BP prior to treatment was on an average 152 mmHg.

All hypotensive agents were withdrawn for at least 8

Table I *Clinical data on the k pertensive patients participating in the study*

	Case no	Age (y)	Sex	Diagnosis	Mean BP (mmHg)	Blood urea (mg 100 ml)	Serum creatinine (mg/100 ml)	Creatinine clearance (ml/min)
Renal failure	1	46	♂	Chronic glomerulonephritis	150	168	18.3	3.1
		53	♂	Polycystic kidney	1 4	166	16.4	3.3
	3	38	♂	Chronic glomerulonephritis	131	250	15.0	8
	4	46	♂	Chronic glomerulonephritis	135	197	10.4	5.3
	5	54	♂	Chronic pyelonephritis	174	162	5.7	16
	6	47	♂	Polycystic kidney	134	91	5.0	15
Mean		47			141	172	11.8	8.5
Essential hypertension	7	62	♂	Essential hypertension	175	73	2.0	53
	8	45	♂	Essential hypertension	130	50	1.6	84
	9	37	♂	Essential hypertension	166	46	1.5	72
	10	42	♂	Essential hypertension	173	38	1.2	105
	11	39	♂	Essential hypertension	158	36	1.0	86
	12	59		Essential hypertension	134	39	0.9	78
	13	32	♂	Essential hypertension	128	41	0.7	132
Mean		43			152	46	1.3	87

da prior to the study The patients were kept on moderate salt restriction, in addition the patients ith renal failure received low protein diet.

The ge distribution as similar in the tw groups. Group I consisted of only males, hereas 6 males and 1 female ere included in group II. Cases ith congestive heart failure or malignant hypertension were not included in the study Furthermore patients recently treated ith rauwolfia alkaloids, guanethidine and long acting diuretic ere xcluded.

The BP as ecorded by the same observer with the use of tandard arm-cuff All patients were examined twice daily (t 11 a.m. and 3 p.m.) in recumbent and upright position, and following moderate exercise. The mean as calculated as the diastolic pressure plus one third of the pulse pressure. All values in the control period (8 days and in the period of treatment ere averaged the average values for the individual patient of systolic, diastolic and calculated mean BP are recorded in Table III.

At the start of treatment 200 mg of l-α-methyldopa (Dopamet®—freeze dried substance dissolved in 45 ml 0.9 saline immediately before use) was given i.v. The supine BP as measured at regular intervals during the following 1 hours. From the next day on constant dose of l-α-methyldopa (Dopamet® tablet) was given orally in tw equal portions at 7 a.m. and 7 p.m. The oral dose had to be reduced after a few days in two cases, in the others the dose was kept unchanged throughout the study

The serum concentration of unconjugated methyldopa as determined following the i. injection, and on days 3 7 8 and 10 after the start of oral therapy Blood samples ere dra n 4 hours after the morning drug dose. (For patients and 3 serum concentrations on days 7 and 8 only ere available.) The means of the serum concentrations found on days 3 to 10 were used to express the verage serum concentration during oral treatment. The drug concentration was determined spectrophotofluorometrically by a method described in detail elsewhere (9)

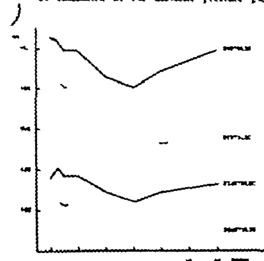


Fig 1 Supine BP after injection of 200 mg methyldopa in 7 patients with essential hypertension (—) and 6 cases ith hypertension and advanced renal failure ( ).

RESULTS

Following i.v. injection of 200 mg methyldopa the serum concentration of unconjugated drug rose to roughly 10 µg/ml in all cases. The average supine BP response in the group with advanced renal failure and in the patients with essential hypertension is shown in Fig. 1 In renal failure the maximum decrease of the average

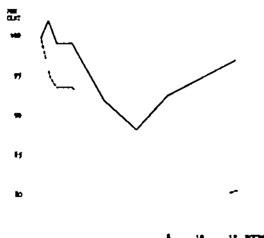


Fig. 2. Changes of supine mean BP (% of control value) in patients with essential hypertension (—) and renal failure (- -) following i.v. injection of 200 mg methyldopa.

systolic and diastolic BP was reached about 8 hours after the drug injection. In the group with essential hypertension the maximum response occurred after about 6 hours for both average systolic and diastolic BP. Calculated in this way the average mean BP showed a maximum fall amounting to 21.5% in the group with advanced renal failure and 11.5% in the patients with normal or slightly reduced renal function, as is shown in Fig. 2.

Table II shows the maximum supine BP response in the individual cases. In many of the patients, however, the maximum reduction was not reached at the same time for both systolic and diastolic BP. Considered in this way, the average maximum decrease of the systolic and the diastolic BP in patients with azotemia was 25 and 20% respectively. In the patients with essential hypertension the reduction was 15 and 14% respectively. The mean BP showed an average maximum decrease of 22 and 14% in the two patient groups.

A distinct sedative effect was observed following i.v. injection of methyldopa, about half of the patients falling asleep during the first two or three hours. No other adverse effects were seen.

A special case, not included in the tables and figures, may be mentioned separately. A 54-year old female with total anuria, caused by a subacute glomerulonephritis, had undergone regular hemodialysis for weeks. She was given 200 mg methyldopa i.v. about 16 hours after the completion of hemodialysis. The BP was recorded in the following 48 hours, during which no hemodialysis was given. The serum creatinine concentration rose from 8.3 to 16.5 mg/100 ml. The supine BP fell from 160/90 to 75/55 about 4 hours after the injection. Despite the marked fall both of systolic and diastolic BP there were no manifest signs of circulatory disturbance. Af-

Table II. *Supine BP response following 200 mg i.v. methyldopa*

	Case no.	Systolic: Before injection (mmHg)	Systolic: Maximum decrease (mmHg)	Systolic: Maximum decrease (%)	Diastolic: Before injection (mmHg)	Diastolic: Maximum decrease (mmHg)	Diastolic: Maximum decrease (%)	Mean: Before injection (mmHg)	Mean: Maximum decrease (mmHg)	Mean: Maximum decrease (%)
Renal failure	1	190	45	24	100	15	15	130	23	18
	2	150	20	13	85	5	6	107	10	9
	3	160	45	28	100	25	25	120	32	27
	4	165	40	24	110	30	27	128	33	26
	5	205	50	24	125	15	12	152	27	18
	6	180	65	36	120	40	33	140	48	34
Mean		175	44	25	107	22	20	130	29	22
Essential hypertension	7	220	45	20	135	30	22	163	35	21
	8	135	25	19	95	15	16	108	18	17
	9	210	15	7	135	5	4	160	7	4
	10	225	25	11	135	15	11	165	18	11
	11	190	15	8	120	15	13	143	15	10
	12	165	55	33	95	25	26	118	35	30
	13	150	15	10	100	5	5	117	9	8
Mean		185	28	15	116	16	14	139	20	14

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THE EFFECT OF METHYLDOPA ON RENAL FUNCTION IN PATIENTS WITH RENAL INSUFFICIENCY

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Abstract. Renal function studies have been performed before and during treatment with methyldopa in ten patients with different degree of renal functional impairment. Effective renal plasma flow increased during treatment in patients both with slight and advanced renal failure. However the glomerular filtration rate decreased slightly in several cases despite increased renal plasma flow. The renal hemodynamic effects of methyldopa could not be related to the serum concentrations of unconjugated methyldopa. The present study suggests that methyldopa is a useful drug for treatment of hypertension also in patients with impaired renal function. In patients with advanced renal failure the treatment should be given with caution because a further decrease of the glomerular filtration rate might occur.

The renal hemodynamic effects of methyldopa have been studied after intravenous administration (7) and short-term peroral treatment (8) in patients with essential hypertension. Increase of effective renal blood flow (RBF) and glomerular filtration rate (GFR) has been reported. This increase is not attributed to changes in cardiac output (7, 8).

Few data are available relating to renal function studies during treatment of hypertension with methyldopa in patients with renal insufficiency. Patients with renal failure are often very sensitive to methyldopa, and hypotension may occur during treatment with low doses of the drug (7, 8). However it is commonly accepted that methyldopa is one of the safer antihypertensive drugs in patients with renal failure (7, 8).

The purpose of the present study was to evaluate the effect of methyldopa on the renal function in patients with advanced renal failure. It is of considerable practical value to know whether the effect on the renal function in patients with severe renal failure differed from that seen in essential hypertension.

MATERIAL AND METHODS

Ten patients with different degrees of renal insufficiency were studied. The diagnosis, age, sex and serum creatinine levels are presented in Table I. Three patients had advanced renal failure. The diagnosis in seven patients was essential hypertension with secondary renal vascular lesion, three patients had primary renal disease with hypertension.

Hypotensive agents were withdrawn for at least 8 days prior to the study. No patient had been treated with rauwolfia alkaloids, guanethidine or long-acting diuretics.

Renal function studies were performed before the treatment with methyldopa (Dopamet®) was started and after 9 days of treatment with the drug. GFR was determined as clearance of inulin (C_{inulin}) and creatinine ($C_{creatinine}$). Effective renal plasma flow (ERPF) was measured as clearance of PAH (C_{PAH}). Inulin was determined by the method of Schreiner (9) and PAH by the method of Finkelstein et al. (3).

RESULTS

The results of the renal function studies are presented in Table II. The three patients with primary renal disease had C_{inulin} below 15 ml/min and C_{PAH} varying from 56 to 200 ml/min. In six of the patients with essential hypertension both C_{inulin} and C_{PAH} were significantly reduced, in two of the patients C_{inulin} was below 50 ml/min and C_{PAH} below 200 ml/min.

After treatment with methyldopa for 9 days the C_{PAH} increased in all but two patients, one with subnormal and one with a high C_{PAH} prior to treatment. Even in patients with a markedly reduced C_{PAH} before treatment a significant in-



Dopamet

ℓ α metyldopa

Om Andersson behöver	½ + ½	= 1 tabl
Petersson	1½ + 1	= 2½ tabl
Lundström „	2 + 2	= 4 tabl
		= 7½ 3 = 2½

och genomsnittsdosen alltså blir 2½ tabl kan man då säga att det bara är Petersson som är välanpassad?
Vad säger Andersson och Lundström om det?
Ingenting – dom har redan talat med sin läkare

dosera individuellt
dosera 2 gånger per dag
utnyttja delbarheten

DUMEX

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PLASMA TURNOVER OF METHYLDOPA IN ADVANCED RENAL FAILURE

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Abstract. Methyldopa is one of the most useful drugs for treatment of hypertension in patients with reduced renal function. Few data are vailable on the metabolism of this drug in renal failure, therefore the plasma turnover rate has been determined in 8 patients with advanced renal failure and 11 cases with essential hyper tension with no or only slightly reduced renal function. In both groups of patients a biphasic plasma elimination curve as obtained after i. injection of the drug. The first, and quantitatively most important, part of the curve showed half-life of 3.6 hours in advanced renal failure compared to 1 7 in essential hypertension. A fairly good correlation was *demonstrated between the half-life of* the drug in plasma according to the initial disappearance rate and the endogenous creatinine clearance. In patients th renal failure only 50–60% of the drug injected was eliminated during the initial phase, the remainder disappeared even more slowly. The slow second phase of the elimination curve was also exponential with slope corresponding to T/2 of 7–16 hours. In patients with normal or nearly normal renal function about 95% of the drug as eliminated during the rapid, initial phase. Our findings are in good agreement with the drug accumulation *occurring in renal failure.*

Patients with renal failure are often very sensitive to the hypotensive effect of methyldopa (3 8). The general opinion is that the higher drug sensitivity in renal failure is due to drug ac cumulation. Few experimental data, however are available on this topic.

The aim of this work was to study the influence of *impaired renal function* on the elimination of methyldopa from plasma. Therefore the plasma turnover rate was determined in one group of patients with renal failure and another group with essential hypertension without renal failure.

MATERIAL AND METHODS

All patients were studied in hospital. All drugs were withdrawn *for at least eight days prior* udy Patients treated with rauwolfia alkaloids, guanethidine or long-acting diuretics ere excluded One patient (case 1) as given hemodialysis 16 hours before and 7? hours af ter the start f the study

The group ith renal failure consisted of eight patients with renal disease associated with hypertension, their aver age serum creatinine concentration being 11.6 mg 100 ml (range 5.0–20) and endogenous creatinine clearance 6 ml min (range 0–16).

Eleven patients with essential hypertension ere examined, the average serum creatinine concentration in this group being 1.. mg/100 ml (range 0 7–..9) and creatinine clearance *98 ml min (range 53–132). A slightly reduced* renal function as present in five of these cases. The age and sex distributions were similar in the tw patient groups.

In 10 of the 19 patients inulin and PAH clearance measurements were performed prior to and few days af ter the study Table II shows that there was good agreement between the inulin and creatinine clearance.

There was no unconjugated methyldopa in plasma at the start of the study The drug concentration as deter mined / 1, .., 4, 6 and 8 hours after i. injection of 200 mg l-α-methyldopa (Dopamet® pro injectione) In eight cases the drug concentration in plasma was also measured after 12, 24, 36 and 48 hours. In addition the serum concentration of unconjugated methyldopa was determined in six patients with renal failure and seven with essential hypertension after eight days of oral treatment with fixed dose of the drug (Dopamet®).

The serum concentration of unconjugated methyldopa (and possibly methyldopamine) was determined by modification of the method developed by Sjoerdsma et al. (7) and Schlossmann et al. (6).

In principle, methyldopa and methyldopamine (unconjugated and the conjugated released by acid hydrolyses) were oxidized to highly fluorescent trihydroxyindole derivatives.

The assay was performed by mixing 1 00 ml of serum with 2.0 ml of distilled water and 0.5 ml of cold 1N perchloric acid. The test tube was placed in icewater for 30 min and centrifuged.

Exactly 2.00 ml of the supernatant was transferred to 10 ml graduated test tube and neutralized with potassium carbonate (10%) using methyl red as indicator (pH approx. 6). *1.0 ml of* phosphate buffer with pH 6 0 and

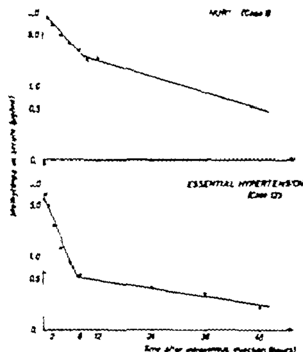


Fig. 1. Representative plasma elimination curves obtained after i.v. injection of 200 mg methyldopa. Case 1, patient with advanced renal failure. Case 12, patient with essential hypertension and nearly normal renal function.

4 ml of distilled water were added, and the sample was oxidized by addition of 200 µl 0.25% (w/v) of potassium ferricyanide. The tube was shaken (Whirli mixer) and kept at room temperature for exactly 5 min. 1.0 ml of a freshly prepared mixture of 9 parts 5% sodium hydroxide and 2 parts % ascorbic acid (w/v) were added.

The tube was shaken (Whirli mixer) and left at room temperature for 1 h 45 min. The tube was shaken now and then (4 to 5 times). The fluorescence emitted at 500 nm was read in a spectrophotometer (Aminco Bowman) during activation of the sample at 400 nm. Reagent blanks, serum blanks, and reference standards with known amounts of methyldopa in serum, were run parallel with the samples.

The recovery of methyldopa carried through the entry procedure was complete (99±6%). The standard error of the mean between duplicate determinations was 10%.

RESULTS

Following i.v. injection of 200 mg methyldopa the serum concentration rose to 8–10 µg/ml, corresponding to an even distribution in a volume of about 20 l. The plasma concentration fell exponentially during the next 6 to 8 hours in all cases the regression line was calculated mathematically and fulfilled the criteria of a first order function. From 6 to 8 hours the elimination curve flattened out this second, slower part of the curve fitted a new exponential function. Thus the elimination of methyldopa from plasma could

Table I. *The elimination of methyldopa from plasma following i.v. injection of 200 mg of the drug in 4 patients with advanced renal failure and 4 with essential hypertension*

Case no	Creatinine clearance (ml/min)	Time interval (h)	Equation of the regression line	Coefficient of correlation	Half-life of drug in plasma (h) Slope 1	Slope 2	Extrapolation of slope 2 to zero time (% of the initial concentration)
1	Anuria	½–8 9–48	log y = 0.9918 − 0.0657x log y = 0.5807 − 0.0185	0.98 0.96	4.58	16.27	38
3	3	½–8 9–48	log y = 0.9447 − 0.0781x log y = 0.6823 − 0.0378	0.99 1.00	3.85	7.97	43
6	6	½–8 9–48	log y = 0 8_34 − 0.0778x log y = 0.4870 − 0.0265x	0.99 0.99	3.87	11.37	40
7	16	½–8 9–48	log y = 1.031 − 0.0733x log y = 0.7490 − 0.0435	0.99 1.00	4.1	6.92	55
12	99	½–8 9–48	log y = 0.8037 − 0.1433 log y = −0.21_5 − 0.0095x	0.98 0.96	_.10	31.47	6
15	108	½–8 9–48	log y = 0.8873 − 0.2144 log y = −0.3048 − 0.0577x	0.99 1.00	1.40	5.22	5
17	131	½–8 9–36	log y = 0.8333 − 0.1948 log y = −0.3887 − 0.0371x	0.99 0.99	1.55	8.11	4
18	132	½–8 9–48	log y = 0.7260 − 0.1611x log y = −0.4533 − 0.0007	0.97 0.36	1.87	(430?)	5

Table II. *Renal function and plasma turnover rate of unconjugated methyldopa in patients with renal failure and essential hypertension*

Case no.	Creatinine clearance (ml/min)	Inulin clearance (ml/min)	T/2 of methyldopa in plasma (h)	Plasma concentration of unconjugated methyldopa (µg/ml): 24 h after 200 mg i.	Plasma concentration of unconjugated methyldopa (µg/ml): Per 100 mg oral drug given for 7 d. or more
Renal failure					
1	Anuria		4.58	1.02	—
2	3	3	3.25	—	0.23
3	3	—	3.85	0.99	—
4	8	7	2.60	—	0.34
5	5	4	3.52	—	0.12
6	6	—	3.87	0.68	—
7	16	—	4.12	0.53	0.26
8	15	[illegible]	2.75	—	0.22
Mean	6	—	3.57	0.71	0.24
Essential hypertension					
9	53	42	2.02	—	0.17
10	84	69	1.67	—	0.19
11	72	—	1.70	—	0.34
12	99	—	2.10	0.37	—
13	105	90	1.63	—	0.27
14	86	97	1.20	—	0.14
15	108	—	1.40	0.02	—
16	78	74	1.90	—	0.13
17	131	—	1.55	0.05	—
18	132	—	1.87	0.32	—
19	132	195	1.27	—	0.22
Mean	98	—	1.67	0.19	0.22

be described by two separate exponential functions as shown in Fig. 1. Because of small concentrations the exact slope of the second part of the elimination curve was somewhat uncertain.

Biphasic plasma elimination curves were observed both in renal failure and essential hypertension without impaired renal function. In normal renal function the slow second phase accounted for the removal of only about 5% of the drug, whereas 90–95% was eliminated during the initial, rapid phase. In renal failure the situation was different, by extrapolation to zero time it could be shown that 40 to 50% of the drug was eliminated from plasma during the slow second phase. Table I shows that the T/2 of the initial part of the elimination curve (slope 1) differed markedly between patients with impaired and normal renal function, whereas the T/2 of the second part (slope 2) did not differ in the two groups.

Table II shows the relationship between glomerular filtration rate (GFR) and the half-life of unconjugated methyldopa in serum as calculated from the initial slope of the elimination curve. In patients with advanced renal failure the half life of the drug in plasma was markedly prolonged, the T/2 being twice that found in patients with a normal or slightly reduced renal function.

Even in the patient with anuria (case 1) there was a significant elimination of unconjugated methyldopa from plasma in fact, the disappearance rate from plasma was not so much slower than that seen in advanced renal failure (Table I).

Fig. 2 shows the relationship between the half life of methyldopa in plasma and the GFR as predicted from the endogenous creatinine clearance. However statistical evaluation of the correlation was somewhat uncertain because the patients were few and grouped in two quite different populations.

In patients with renal failure there was a considerabl retention of unconjugated methyldopa in plasma 24 hours following i.v injection of 200

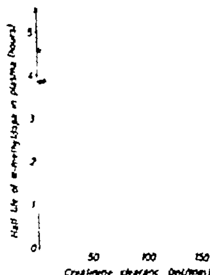


Fig. The relationship between the half-life of unconjugated methyldopa in plasma (slope 1) and GFR. O = patients with renal failure, • = patients with normal or slightly reduced renal function.

mg of the drug (Table II). The drug retention in plasma amounted to 3–4 times that observed in patients with normal renal function. Surprisingly the serum concentrations of unconjugated methyldopa achieved after seven days of oral therapy with a fixed dose did not seem to be much influenced by the GFR (Table II).

DISCUSSION

... cepted that methyldopa is mainly ... he kidneys and that drug reten ... n renal failure (3). However ... e the elimination of the drug ... died in advanced renal failure or in ...

The p ... learly demonstrated that the eliminati ... on ugated methyldopa was significantly ... n patients with renal failure. The half-li ... conjugated methyldopa in plasma of pa ... ith advanced renal insufficiency was pr ... nged to more than twice that found in cases with essential hypertension and a relatively well preserved renal function. Our figures obtained in normal renal function were in good agreement with data given by others (7). The prolonged half-life of the drug in plasma was also reflected in the d ... g concentration achieved 24 hours after the i.v. injection in renal failure significantly elevated levels were observed. The practical implication of these observations is that accumulation of methyldopa might occur in patients with renal insufficiency because of slower drug elimination; therefore the drug dose should be adjusted according to the renal function in such cases.

We observed a biphasic plasma elimination curve in all cases, irrespective of the glomerular filtration rate. In spite of rather low plasma concentrations, the finding was valid. The exact calculation of the second part of the elimination curve was, however, somewhat uncertain. In patients with normal renal function the slow second part of the elimination curve accounted for removal of about 5% of the drug injected. In advanced renal failure, however, this second phase of elimination started at plasma concentrations of 40–50% of the initial value. Thus the slow phase of the elimination process was quantitatively much more important in renal failure than in normal renal function. In principle, the plasma elimination curve obtained in one patient with anuria was quite similar to those found in advanced renal failure.

The cause of the biphasic plasma elimination curve is not known. Such a pattern might be expected if some of the drug was bound to plasma proteins. However, in agreement with others, we found no protein-binding of unconjugated methyldopa (2, 9). An inflow of drug into plasma after 6 to 8 hours does not seem probable, since there was a complete equilibration between plasma and extravascular compartments a few minutes after the i.v. drug injection. The drug concentration achieved shortly after the injection suggested an even distribution of the drug in an extravascular fluid volume of 20 to 25 l irrespective of the renal function. Inflow of drug from extravascular sites would be expected to occur much earlier than after 6 to 8 hours, and it could not explain the observed difference between normals and patients with renal failure. We have observed a higher proportion of methyldopa to be conjugated in azotemia (5), but the drug conjugation is a rather slow process which may not influence the actual elimination curves. Another possibility is that the second slope of the plasma elimination curve could be due to drug-induced reduction of the renal function, though we did not find depression of the GFR or renal plasma flow during

short-term treatment with methyldopa (1). A self-depression of a renal transport mechanism seems unlikely since the excretion of unconjugated methyldopa is mainly a result of glomerular filtration (4).

The most probable explanation of the biphasic plasma elimination curves is that extrarenal drug elimination occurs, and that this mechanism is most important at high plasma drug levels. In fact we have found strong indications of such a mechanism (4). Extrarenal elimination of unconjugated methyldopa might also explain the drug elimination in the case of anuria. In the patient with anuria we also observed a biphasic elimination curve. The initial slope was retarded to about three times the normal, and the second phase of the elimination started at a plasma level of 40% of the initial drug concentration. In other words, the drug elimination in anuria was surprisingly good and could only be explained by extrarenal drug elimination.

In conclusion, this study clearly demonstrated impaired elimination of methyldopa in renal failure, the half-life of unconjugated methyldopa in plasma being proportional to the decrease in GFR. The plasma elimination curve was biphasic, suggesting two different mechanisms in the process of elimination. Further study is demanded to elucidate this problem.

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HYPOPHYSEO-ADRENOCORTICAL FUNCTION IN DIABETES MELLITUS

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Abstract. Earlier experiments have given conflicting information on the hypophyseo-adrenal function in diabetic patients. Normal suppression of plasma corticosteroids with 1 mg of dexamethasone (1 mg-DST) in stable, non-insulin-treated diabetics has previously been reported. However, only a partial response was found during 1 mg-DST in approximately one-third of the juvenile, insulin-treated diabetics, indicating a slight hypercorticism in these patients. The cause of this was presumed to be repeated small stress-stimuli as a consequence of fluctuations in the blood sugar. The present study attempts to elucidate this supposition. In contrast to earlier investigations a normal circadian rhythm and normal baseline values of plasma corticosteroids were found in 19 insulin-treated diabetics during "basal" conditions, but this does not exclude the presence of a slight hypercorticism in some of the patients. Plasma corticosteroids and blood sugar were determined during 96 subjective hypoglycaemic attacks in 73 insulin-treated diabetics. Elevated values of plasma corticosteroids were found both in cases of normal and low values of blood sugar. In six insulin-treated diabetics with hyperglycaemia and ketonuria (four without acidosis) elevated values of plasma corticosteroids were found. The results support the assumption that a fall in the blood sugar to low values and fluctuations in the blood sugar at normal or high levels can bring about increased hypophyseo-adrenal activity and that this might be the cause of a slight hypercorticism in some cases of juvenile, insulin-treated diabetics. It is not known whether the presumed hypercorticism is a reversible condition or not.

In 1945 Russi et al. (22) demonstrated an increased frequency of adrenal cortex adenomas in diabetics; this finding has later been confirmed (4, 11). Becker (3) and Becker et al. (4) observed a more frequent occurrence of fatty vacuolation in zona fasciculata, a higher frequency of adrenal cortex adenomas and a greater weight of the adrenal glands in diabetics with nephropathy than in diabetics without nephropathy. From these observations it was presumed that diabetics had an increased corticoid production, and that there was a pathogenetic relation between the hypercorticism and the development of angiopathia diabetica (3, 4).

However, determinations of corticosteroids in plasma or of corticoid metabolites in the urine have given conflicting information about the function of the adrenal cortex in diabetics. Normal baseline values of urinary 17-hydroxycorticosteroids (17-OHCS) (12, 16, 18, 21, 24), urinary 17 ketogenic steroids (17-KGS) (1) and normal baseline values of plasma 17-OHCS (12, 1 24) have been found in diabetics with and without angiopathy. In the above investigations the patients were selected regardless of whether they received insulin or not; blood sugar level was fairly well regulated. Serio et al. (23) found normal circadian rhythms and normal baseline values of fluorimetrically determined plasma cortisol in eight non-insulin-treated diabetics with satisfactory blood sugar regulation.

In diabetics with and without angiopathy a normal adrenal cortex response has been observed during the corticotropin test (ACTH-test) (12, 18, 21). In eight insulin-treated diabetics with a satisfactory blood sugar regulation Eik Nes et al. (7) found a normal increase in plasma 17-OHCS during a 6-hour maximum ACTH-stimulation test.

However, other investigations have indicated that the adrenal activity is increased in certain diabetics. In diabetics with angiopathy Lentle and Thomas (15) found an increased adrenal cortex activity. Their material comprised 15 diabetics, six without and nine with angiopathia diabetica; two patients in each group did not receive insulin. In both groups elevated baseline values of

plasma 17-OHCS were found at 2 p.m. and 3 a.m. In diabetics with angiopathy there was an absence of circadian rhythm of plasma 17-OHCS significantly increased urinary 17-OHCS and a significantly elevated cortisol secretion rate. Of five diabetics with angiopathy two had no and three an incomplete suppression of urinary 17-OHCS during the administration of 8 mg dexamethasone daily for four days. During a 3-day ACTH-test the response in urinary 17-OHCS was elevated in diabetics with angiopathy and normal in diabetics without angiopathy. During a 4-hour ACTH-test and during a pyrogen test an abnormally high response was found in plasma 17-OHCS.

Asfeldt (1) carried out a 1 mg dexamethasone suppression test (1 mg DST) in diabetics and found normal suppression of plasma cortico-[illegible]oids in 16 non-insulin treated stable diabetics [illegible]hout angiopathy and with well regulated blood sugar levels. In 15 of 41 insulin-treated [illegible]venile diabetics an incomplete response was found during 1 mg DST. Compared with the results in normal responding juvenile diabetics, the mean 8 a.m. baseline [illegible]alu[illegible] of plasma cortico-ste[illegible]oids was significantly h[illegible]gh[illegible] and the decrease in plasma corticost[illegible] during 1 mg-DST was significantly sma[illegible] [illegible]n[illegible]ompletely responding juvenile diabe[illegible] [illegible]ve with 2 mg dexamethasone p[illegible] [illegible]ticosteroids were suppressed [illegible] (1) but this amount of [illegible]examethas[illegible] known to give insufficient [illegible]sponse [illegible] [illegible]hing[illegible] [illegible]ndrome (1[illegible] 17). The partial [illegible] 1 mg DST was not dependent on the p[illegible] [illegible]f diabetic angiopathy. With rega[illegible] [illegible] du[illegible] [illegible]on of diabetes total 24-hour insulin dosa[illegible] [illegible]nd [illegible]tal [illegible]4-hou[illegible] urinary sugar excretion no [illegible]gni[illegible]cant diffe[illegible]ence could be de[illegible]monstrated betwe[illegible]n partial [illegible]nd normal responders.

Asfeldt (1) proposed the hypothesis that the presumed slight hype[illegible]corticism in about one third of the juven[illegible]l[illegible] diabe[illegible] was due to repeated small stress-stimul[illegible] [illegible]n the form of fluctuations in the blood suga[illegible] level. Earlier observations support this hypothesis.

Jaumann (13) found [illegible]n [illegible]ncrease in plasma 17-OHCS in five diabetics during insulin-induced fall in the blood sugar le[illegible]el despite the fact that the lowest blood sugar level was no lower than 9[illegible]–177 mg/100 ml, the [illegible]ate of fall of the blood sugar was, however, extremely rapid.

Göth et al. (9) found an increased urinary corticoid excretion and elevated values of plasma 17-OHCS in eight insulin-treated diabetics with relatively high blood sugar levels and large fluctuations in the blood sugar but without ketoacidosis. In 11 well regulated diabetics with relatively minor fluctuations in the blood sugar and with relatively low blood sugar levels, a normal urinary corticoid excretion and normal baseline values of plasma 17-OHCS were found.

In a particularly well controlled trial, Bolinger et al. (5) found significantly higher excretions of urinary 17-OHCS in five juvenile diabetics in a period with strongly varying blood sugar at a relatively high level than was the case in a period with minor fluctuations in the blood sugar at a lower level; the glucosuria was not particularly high in either of the periods.

Hyperglycaemia with ketoacidosis, during which the adrenocortical activity is increased (1[illegible], 14, 24, 25) occurs relatively rarely in insulin-treated diabetics. On the other hand considerable fluctuations in the blood sugar undoubtedly occur daily in many insulin-treated juvenile diabetics, which might well be the cause of an increased pituitary adrenocortical activity in such patients.

In the present study this supposition is elucidated by determining the plasma corticosteroids in juvenile insulin-treated diabetics during "basal" circumstances, in patients with subjective sensations of hypoglycaemia as well as in patients with hyperglycaemia with or without ketoacidosis.

MATERIAL AND METHODS

Unconjugated plasma corticosteroids were determined fluorimetrically (19). By this technique plasma 11-hydroxycorticosteroids (cortisol + corticosterone) are determined.

Blood and urinary sugar were estimated according to the method of Hagedorn et al. (10).

I. Baseline [illegible]alues and circadian rhythm of plasma corticosteroids during "basal" conditions

The material comprised nine patients with angiopathia diabetica (retinopathia and/or nephropathia diabetica) and possibly also with neuropathia diabetica (group A) and ten patients without demonstrable diabetic complications (group B). The patients received insulin once or twice daily and [illegible] balanced diet; none had acetonuria or subjective sensation of hypoglycaemia on the day of the investigation. I[illegible] selecting the patients no regard has been paid to th[illegible] regulation of the blood sugar. Table I summarizes the patients' clinical and biochemical data. None of the patients in group B had proteinuria. Mean values

Table I. *Clinical and biochemical information on 19 juvenile diabetics participating in the study*

Case no.	Sex	Age (y.)	Duration of diabetes mellitus (y.)	Retino-pathy	Nephro-pathy	Neuro-pathy	Serum creatinine (mg/100 ml)	Urinary sugar (g/24 h)	Insulin (IU/24 h)	Range of blood sugar (mg/100 ml)
Group A										
1	♀	69	34		+		1.44	traces	4+12	80–295
2	♀	22	14	+			1.20	48	36+4	185–70
3	♀	52	27	+		+	1.38	traces	8	70–310
4	♂	24	17	+	+	+	1.46	34	28 6	110–90
5	♂	52	29	+	+	+	0.62	62	24	23 324
6	♂	36	30	+			1.16	65	28−4	180–325
7	♂	36	15	+	+	+	1.00	18	20−12	190–310
8	♂	66	7	+		+	1.18	32	44−8	145–5
9	♂	48	36	+		+	1.26	8	16 10	60–235
Mean		45.0	22.1				1.19	29.7	33.8	
S.E.M.		5.6	3.1				0.27	6.7	5.1	
Group B										
1	♀	20	0.1				0.74	54	24−8	170–290
2	♀	63	15				0.92	19	56−12	150–360
3	♂	21	10				0.82	59	40+8	70–340
4	♂	20	5				?	19	34−18	75–165
5	♂	38	1				0.80	24	28+12	150–315
6	♂	37	5				?	6	32 16	60–250
7	♂	25	0.5				1.27	traces	16	100–200
8	♂	25	5				?	traces	20	85–175
9	♂	36	6				?	43	28−8	85–40
10	♂	38	10				1.17	70	20−12	155–310
Mean		32.3	5.8				0.95	29.4	39.0	
S.E.M.		4.3	1.5				0.28	6.7	4.8	

of serum creatinine in group A was significantly higher than in group B ($p < 0.001$). No significant difference was found between mean values of urinary sugar/24 h ($p > 0.1$) or between mean values of 24-hour insulin dosage ($p > 0.1$).

Heparinized venous blood samples were drawn at 5 a.m., 7 a.m., 9 a.m., 12 a.m., 7 p.m. and 10 p.m. of the same day for plasma corticosteroid determinations, and at the same times capillary ear blood was taken for blood sugar determinations.

II. Plasma corticosteroids in diabetics with subjective sensations of hypoglycaemia

All insulin-treated diabetics in the department were requested to inform the nurse, no matter at what time of day, if they had sensations of commencing or manifest hypoglycaemia. The selection rested only on the patient' evaluation of the symptoms, which varied from mild to severe, though without loss of consciousness.

Heparinized venous blood was immediately drawn for the determination of plasma corticosteroids and capillary ear blood was taken for the determination of blood sugar. None of the results have been excluded from the material.

The material comprised 43 patients, 22 men and 21 women (aged 10–74), who did not have angiopathia diabetica, and 30 patients, 20 men and 10 women (aged 17–59), who had angiopathia diabetica (retinopathia and/or nephropathia). The serum creatinine was less than 1.50 mg/100 ml in 24 patients, 2.00–3.00 mg/100 ml in four and 3.36–5.65 mg/100 ml in two patients in the group with angiopathia diabetica. In the group without angiopathia diabetica, serum creatinine was less than 1.50 mg/100 ml. The patients received insulin once or twice daily and a balanced diet.

III. Plasma corticosteroids in diabetics with hyperglycaemia and acetonuria with or without ketoacidosis

The material comprised the following six patients.

Case 1. A 37-year-old man with diabetes mellitus (d.m.) for five years who had been admitted with ketoacidosis. Serum total $-CO_2$ 9.1 mmol/l, blood sugar 405–230 mg/100 ml.

Case 2. A 12-year-old boy with d.m. for three years who had been admitted with ketoacidosis. Serum total $-CO_2$ 9.2 mmol/l, blood sugar 408 mg/100 ml.

Case 3. A 16-year-old boy with d.m. for 4 years who was admitted with hyperglycaemia, ketonuria and pneumonia. Rectal temperature 40.4–38.8°C, serum total $-CO_2$ 25.3 mmol/l, blood sugar 245–175–125–125 mg/100 ml.

Case 4. A 27-year-old man with d.m. for two months who was admitted with hyperglycaemia and ketonuria.

hypercorticism in several of the patients at the moment of the investigation. Thus, in Cushing's syndrome, both normal baseline values and a preserved circadian rhythm have been observed (8) It would have been desirable that the 1 mg DST had been carried out in these patients, so that the circadian rhythm in partial responders could have been compared with that of normal responders. In fact, Asfeldt (1) found a significantly higher mean 8 a.m. baseline value of plasma corticosteroids in partial responders.

During attacks with subjective sensation of hypoglycaemia a number of patients had elevated values of plasma corticosteroids despite the fact that the blood sugar was not always low In these cases a rapid fall in the blood sugar must presumably be the cause of the symptoms and of an elevation of plasma corticosteroids this is in agreement with earlier observations (5 9 13) Elevated values of plasma corticosteroids were also found in diabetics with hyperglycaemia without acidosis. This observation is in agreement with that of Góth et al. (9)

Thus it is likely that large fluctuations in the blood sugar at normal and high levels and a rapid fall in the blood sugar to a hypoglycaemic level will bring about an increased pituitary-adrenocortical activity Hyperglycaemia or other parallel metabolic disturbances in poorly regulated diabetics apparently also increase the pituitary-adrenocortical activity

An increased pituitary-adrenocortical activity may be present in certain diabetics. In support of this supposition, emphasis is to be placed on the finding of an increased cortisol secretion rate, an increased adrenal cortex response during ACTH test (15) and a partial response during 1 mg DST (1) Asfeldt (1) found partial response during 1 mg-DST in one-third of the patients in the group of diabetics who empirically have the most fluctuating blood sugar levels. The hypothesis (1) that the cause of a presumed slight hypercorticism in these patients was repeated small stress-stimuli in the form of fluctuations in the blood sugar is supported by the present study

The presumed increased pituitary-adrenocortical activity in certain diabetics may be a reversible or a permanent condition The observations of Bolinger et al. (5) indicate that the condition is reversible. Asfeldt (1) carried out the 1 mg DST three times at monthly intervals in a juvenile diabetic and found a partial response each time, viz. an irreversible condition. However 1 mg DST ought to be carried out in a larger series of juvenile diabetics both when the blood sugar is poorly regulated and when it has been well regulated for a suitable period.

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DRUG INDUCED MALABSORPTION OF VITAMIN B_{12}

IV *Malabsorption and Deficiency of B_1 during Treatment with Slow-Release Potassium Chloride*

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Abstract In series of 60 patients on KCl treatment the Schilling test was abnormal (below 5%) in 12% slightly reduced (5 to 10%) in 18% and normal (above 10%) in 70%. Radiotelemetric measurement of the ileal pH in 21 patients showed an acidification of the ileal contents to be the mechanism of the B_{12} malabsorption found. In 10 patients with an ileal pH of 6 or below the Schilling test was on average 7.8% and in those 11 with pH above 6 the corresponding figure was 13.4%. The serum levels of vitamin B_{12} in 17 heart patients taking KCl over period of 6 to 24 months were, however, not significantly lower than those of the control group. Potassium chloride induces malabsorption of vitamin B_{12} in some patients, but in rare instances is the regular potassium supplementation of long enough duration to be the only factor causing the deficiency state of B_{12}.

The malabsorption of vitamin B_{12} caused by para-aminosalicylic acid (4) is most probably due to inhibition of some folate-dependent enzymic system in the wall of the intestine (6). In a preliminary communication (8) we reported our first observations on the disturbed absorption of B_{12} during treatment with slow-release potassium chloride tablets and suggested that the change of pH could be the mechanism of the phenomenon. The present paper reports our further observations on the absorption of B_{12} and the serum level of B_{12} in a larger series of patients taking KCl tablets, as well as studies of the above mentioned pathogenetic mechanism.

MATERIAL AND METHODS

T study the absorption of vitamin B_{12} series of 60 male patients in the Medical Ward were studied, excluding patients with pernicious anaemia, fish tapeworm and other known reasons for B_{12} malabsorption. They received 1.0 g KCl three times daily as slow-release tablets (Kalduron® Orion, Helsinki). After medication for at least three days a Schilling test was performed (9), using 1.0 µg of ^{57}Co B_{12} (Institutt for Atomenergi, Kjeller) as test dose

The group of 46 patients studied for the possibility of developing deficiency of B_{12} were ambulatory heart patients taking digoxin and thiazide diuretics. Seventeen of them ere prescribed Kalduron® as routine measure, the other 29 were control cases. The serum level of B_{12} was assayed using Euglena gracilis strain Z (7).

Measurements of the pH in the small intestine were obtained from 33 patients during potassium medication using radiotelemetric equipment manufactured by Telefunken A.G., Ulm (6). According to the manufacturer the sensitivity of the method is 0.5 pH unit. The measuring capsules were localized radiologically The time between the Schilling test and the measurement of the pH was not more than three days.

RESULTS

Schilling tests

The Schilling test value in the 60 patients during KCl treatment was abnormal (below 5%) in 12% slightly decreased (5 to 10%) in 18% and normal (above 10%) in 70% of the cases. In 15 patients with a low alue the test was repeated without KCl medication. The average alues of the Schilling tests for these patients were 5.1% during potassium chloride treatment and 12.0% without it.

Serum level of vitamin B

The serum level of vitamin B_{12} was measured in 46 heart patients on digoxin and diuretics. Among them 17 were taking regular potassium supplementation. The period of KCl treatment was 6 to 24 months, in most patients less than 12 months. The serum level was, on average, 236 pg/ml in the KCl group (17 pats.) and .68 pg/ml in the

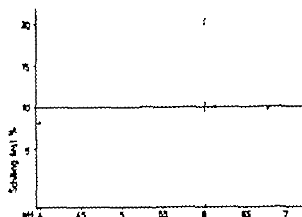


Fig 1 The ileal pH and Schilling test values for 21 patients during KCl treatment.

control group (29 pats.) There were two patients with values under 150 pg/ml in each group. The differences are not statistically significant.

Ileal pH and absorption of B

Radiotelemetric measurements of the ileal pH were performed on 33 patients during KCl medication. In 21 cases the procedure was technically successful, in the other 12 the measuring capsule remained in the stomach for several hours and these cases were excluded. The results of the 21 successful pH measurements in the ileum are compared with the Schilling tests for the same patients in Fig. 1 and Table I. Reduced Schilling test values and low ileal pH were observed for the most part in the same patients. In 10 patients with ileal pH of 6 or below the Schilling test was on average 7.8% while in the 11 patients with an ileal pH above 6 the corresponding value was 13.4%. Table I shows the normalization of the Schilling test values after cessation of KCl.

DISCUSSION

In vitro experiments (1) have shown that the intrinsic factor effect on the uptake of B_{12} by normal ileal homogenate is dependent on the pH in the environment. It has been found to be maximal at pH 6.6 and above and absent below 5.5. The present in vivo results concur with those experiments. At a pH level of below 6 the average value of the Schilling tests was about half of the average value of tests on a pH level of above 6. The overlapping of the results was not greater than could be expected considering the errors of the two methods used.

Potassium chloride as a slow-release tablet has become very popular as a method of preventing the development of hypopotassaemia and hypochloraemic alkalosis during treatment with thiazide diuretics. Potassium chloride is an acid compound (the pH of a 1% KCl solution is 5.1) and in therapeutic dosage it is capable in some patients of rendering the almost neutral content of the ileum so acid that the intrinsic factor activity is inhibited and the absorption of B_{12} is disturbed.

The idea that KCl tablets could interfere with the absorption of B_{12} was obtained from a heart patient with megaloblastic anaemia without any known aetiology (8). She was in too poor a condition for any experiments, but she was an example showing that a deficiency of B_{12} may develop under certain circumstances during KCl treatment. The B_{12} stores in the body are large (3), and the disturbance of B_{12} absorption caused by KCl is not total. Thus KCl as the only factor causing a B_{12} deficiency must be rare, at least it requires regular medication during several years to develop. However, most heart patients with an

Table I. *The ileal pH and Schilling test values for 21 patients during treatment with KCl, and the Schilling tests repeated without KCl in those who had abnormal values*

Case no.	During KCl medication		Without KCl
	Ileal pH	Schilling, %	Schilling, %
1	4 1	8.3	15.0
2	5.2	10.7	11 1
3	5.3	6.8	10.0
4	5.5	6.7	10.5
5	5.5	11.0	16.5
6	5.6	4.3	7.8
7	5 7	1.5	18.0
8	5.9	8.5	12 0
9	5 9	12.5	
10	6 0	6 7	9.0
11	6 1	22 0	
12	6 2	4 9	17 0
13	6.2	10 0	19.0
14	6.2	12.5	
15	6.2	18.0	
16	6.4	11 0	
17	6 5	12 5	
18	6 7	15.5	
19	6 8	9 1	11 3
20	6 9	19.0	
21	7 0	11.0	

indication for KCl supplementation are elderly. The intrinsic factor activity decreases with advancing age (2) and among elderly patients one would expect to find many with a slightly disturbed B_{12} absorption and reduced B_{12} stores. In those already close to a deficiency of B_{12}, a further B_{12} disturbance caused by KCl may be the additional factor leading to a deficiency state. As could be expected from the relatively short period of observation, our results concerning falling serum levels of B_{12} during KCl are not convincing. During long-term treatment with KCl for many years one should, however, remember the possibility of developing B_{12} deficiency as a complication of the drug.

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INTRAVENOUS NUTRITION WITH AMINO ACID SOLUTIONS IN PATIENTS WITH CHRONIC URAEMIA

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Abstract. Studies are reported dealing with nutrition of patients in terminal uraemia by i.v. infusions of solutions of the essential amino acids supplementing a diet with a low standardized nitrogen content. The basic amino acids were neutralized with acetic acid. Most of the patients had a creatinine clearance below 5 ml/min. The nitrogen balance improved in all patients except in a few cases with intercurrent complications. In most cases the plasma urea concentration decreased concomitantly. The beneficial effect of the essential amino acids on the nitrogen balance and the plasma urea was enhanced by the addition of histidine and also of both histidine and arginine to the amino acid solution.

Patients with chronic uraemia are in a catabolic state with a negative nitrogen balance and a decreased muscle mass (21). Additional factors are gastro-intestinal symptoms which contribute to the poor nutritional state. Traditionally these patients are treated with a nitrogen-poor diet in order to decrease the urea nitrogen in the body fluids and to reduce the uraemic symptoms.

A synthetic diet containing the eight essential amino acids as the only source of nitrogen (16) or a nitrogen-poor diet with the addition of essential amino acids given by mouth may bring about a slightly positive nitrogen balance (3, 19). We have examined the effect of treating uraemic patients with the essential amino acids by i.v. infusion (13, 24, 25). This enabled us to treat successfully even patients suffering from nausea and vomiting, as well as patients in the post-operative state.

It has been shown that i.v. administration of amino acids preferentially supplies the peripheral cells, i.e. muscle cells, whereas oral administration in the first place supplies the liver (10, 12, 15, 25).

A study was made of the nitrogen metabolism of patients in the terminal state of uraemia receiving a standardized diet poor in nitrogen. After a period on this diet only (series A) the diet was supplemented with i.v. infusions of the eight essential amino acids either alone (series B) or together with histidine (series C) or with both arginine and histidine (series D). The L-forms of amino acids were used. The nitrogen metabolism was followed by determining the total nitrogen loss in the urine and the concentration of urea nitrogen in the plasma.

MATERIAL AND METHODS

Patients

Fifty-four balance studies were carried out on 20 patients with chronic renal failure and azotaemia. The endogenous creatinine clearance was below 5 ml/min in 18 cases, 5–6 ml/min in the other two. Clinical data, including diagnosis, are presented in Table II. All the patients were informed of the aim of the study and gave their consent to the treatment.

On admission to the hospital the water, electrolyte- and acid-base status of the patients were evaluated and measures were taken to bring the patients into and maintain them in an optimal steady state condition.

Nitrogen-poor diet

When a steady state had been achieved, all patients were given a diet standardized with regard to the nitrogen content (2.4–3.0 g nitrogen/day controlled by occasional Kjeldahl determinations) and providing 2 300–3 000 kcal/day about 70% of which were given as carbohydrates. The diet was supplemented with vitamins and trace elements. In a few cases, who obviously did not follow the dietary regimen, the study was discontinued.

Amino acid solutions

The infused solutions (prepared by Astra, Södertälje, Sweden) consisted of the eight essential amino acids in

Table I. *Amino acid solutions*

	Series B		Series C		Series D	
	g/l	g N/l	g/l	g N/l	g/l	g N/l
L-phenylalanine	8.25	0.70	8.25	0.70	8.25	0.70
L-isoleucine	5.25	0.56	5.25	0.56	5.25	0.56
L-leucine	8.25	0.88	8.25	0.88	8.25	0.88
L-lysine	6.00a	1.15	4.00a	1.15	6.00b	1.15
L-methionine	8.25	0.77	8.25	0.77	8.25	0.77
L-threonine	3.75	0.44	3.75	0.44	3.75	0.44
L-tryptophane	1.88	0.26	1.88	0.26	1.88	0.26
L-valine	6.00	0.71	6.00	0.71	6.00	0.71
L-histidine	0	0	4.12a	1.12	4.12b	1.12
L-arginine	0	0	0	0	8.85b	2.85
Total	47.83	5.47	51.75	6.59	60.60	9.44

a As acetate. b As hydrochloride.

the proportions recommended by Rose (28), with or without addition of constant proportions of histidine or of histidine + arginine (Table I). As a rule the basic amino acids lysine and histidine were neutralized with acetic acid (instead of hydrochloric acid) in order to prevent acidosis. Exceptions from this rule were made in series D 2 (which was, in fact, the first series of observations made) and in one experiment in series D 1 (case 11). Here arginine, histidine and lysine were dissolved as chlorides, thus yielding an excess of protons and tending to produce a progressive metabolic acidosis. This complication was prevented by giving sodium bicarbonate, 6–10 g/day by mouth. Patients with a marked tendency to sodium and water retention were not considered for treatment with the solutions containing chlorides requiring neutralization with sodium bicarbonate.

General arrangement of the balance studies

The amino acid solutions were infused daily over a period of 4 hours or on alternate days over a period of 8 hours when double the dose was given. The nitrogen intake was calculated from the nitrogen content of the diet and of the amino acid solution infused.

The urine was collected in 24-hour portions for determination of total nitrogen. Faecal nitrogen was not determined. The nitrogen balance was calculated without correction for assumed losses in the stools or in the sweat ("apparent nitrogen balance" (14)).

Plasma urea nitrogen (PUN) and creatinine were determined daily or every second or third day during the metabolic studies. A correction of the nitrogen balance

Table II. *Clinical data on all 20 patients, and results from the series A studies in which nitrogen-poor diet was given but no amino acid infusions*

P.C.K. = polycystic kidney disease, C.P = chronic pyelonephritis, C.G = chronic glomerulonephritis, I.N = interstitial nephritis (phenacetine abuse)

	Sex	Age (y)	Diagnoses	Endogen. creatinine clearance (ml/min)	B. wt. (kg)	Total body water (l)	Days	PUN (mg/100 ml) Before	PUN (mg/100 ml) After	Plasma creatinine (mg/100 ml) Before	Plasma creatinine (mg/100 ml) After	App. nitrogen balance (g N/d.) Non-corrected	App. nitrogen balance (g N/d.) Corrected
1	[illegible]	51	P.C.K.	5	75	45	3	148	136	16	14	−3.3	−1.5
2	♀	36	P.C.K.	5	41	32a	6	75	55	10	10	−2.5	−1.1
3	[illegible]	56	C.P	5	74	31	4	105	103	18	18	−1.8	−1.5
4		49	P.C.K.	3	51	28a	5	71	48	12	13	−1.4	−1.2
5	[illegible]	34	P.C.K.	6	58	34a	4	100	88	13	12	−3.5	−2.5
				5	58	34a	4	79	51	11	11	−1.5	0.9
6	♀	37	C.P	<5	45	25	4	156	114	12	10	−4.3	−1.7
				<5	45	25	1	100	99	12	12	−[illegible]	−2.1
7	♀	67	C.P	2	56	31	3	133	115	12	11	−1.4	0.4
8	♂	47	C.G	5	67	40	3	92	75	11	11	−1.6	−1.2
				5	67	40	4	95	71	14	12	−3.3	−0.9
9	♀	40	P.C.K.	5	62	34a	4	104	84	13	14	−2.1	−0.1
10	♀	50	C.P	7	59	32	9	75	34	8	7	−0.9	0.5
11	♀	47	C.G	<5	49	27	12	114	69	12	13	−2.0	−0.9
12	♀	32	C.G	3	56	31	4	111	94	12	12	−3.1	−1.8
13	♂	6[illegible]	C.G.	5	76	45	12	90	59	16	17	−1.5	−0.3
14	♀	44	I.N	4	58	32	8	69	54	12	10	−1.4	−0.8
15	♀	57	C.G	3	72	40	15	74	57	17	17	−0.9	−0.4
16	♀	22	C.G.	3	54	30a							
17	♀	21	C.G	2	32	29a							
18	♂	37	I.N	4	54	33a		Not studied					
19	♀	50	P.C.K.	<5	56	30							
20	♀	49	I.N	5	54	30							

a Determined by tritiated water.

Table III. *Results from the series B patients treated daily with the eight essential amino acids*

Case no.	B. wt. (kg)	Total body water (l)	Days	Amino acids (g N/d.)	PUN (mg/100 ml) Before	PUN (mg/100 ml) After	Plasma creatinine (mg/100 ml) Before	Plasma creatinine (mg/100 ml) After	App. nitrogen balance (g N/d) Non-corrected	App. nitrogen balance (g N/d) Corrected
1	75	45	6	3 7	136	120	16	17	0.8	1.7
2	61	37[a]	7	2.2	55	51	10	10	1 1	1.3
3	74	31	8	2.2	103	83	18	19	1.3	2.1
16	56	30[a]	5	1 1	49	42	11	13	1 1	1.5
17	52	29[a]	9	2.2	57	61	13	12	1.6	1 4

Determined by tritiated water.

was made for changes in the total urea pool (urea nitrogen concentration total body water volume) ("corrected apparent nitrogen balance"). Changes of other non-protein nitrogen substances were not considered when the corrected balances were calculated.

With three exceptions balance studies were not performed in patients with severe complications.

Analytical methods

Blood glucose was determined by the ortho-toluidine method (23), urine nitrogen by the Kjeldahl method, insulin (32) and HGH (human growth hormone) (5) by immunological agglutination methods (by Dr E. Cerasi, Dept. of Endocrinology and Metabolism, Karolinska Sjukhuset, Stockholm) and PUN and creatinine by methods previously reported (11). Total body water volume was determined by isotope dilution using tritiated water or was assumed to be 60% of the b.wt. in male patients and 55% in female.

Series of balance studies

Fifteen patients (series A, Table II) were studied for 1 to 12 days (in most cases 4–6 days) when on the diet alone, before being given amino acids by i. infusion.

In three cases (case 16, Tables II, III and V case 17 Tables II, III, IV and V and case 19 Tables II and V) the amino acid infusions were given from the start of the diet. In tw cases (case 18, Tables II and IV and case 20, Tables II and VI) the study was not started until after few weeks of amino acid treatment.

Five patients (series B, Table III) were given only the eight essential amino acids by daily i. infusions. The daily dose of essential amino acids corresponded to 2.2 g nitrogen/day except in two cases who received 1 1 and 3 7 g nitrogen/day respectively

Seven patients (series C, Table IV) were given essential amino acids (2.2 g nitrogen) supplemented with an amount of histidine corresponding to 0.45 g nitrogen included in the daily infusions. One patient in this series received double the dose of essential amino acids and histidine daily in one of his three periods of treatment.

Eleven patients (series D Table V) were given both arginine (1.12 g nitrogen) and histidine (0.45 g nitrogen) in addition to the essential amino acids (2.2 g nitrogen). The amino acids ere infused daily in eight patients (D 1) or were given as double dose every second day in five patients (D 2).

Three patients (series E, Table VI) ere studied in

Table IV *Results from the series C patients treated daily with the eight essential amino acids and histidine*

Case no	B. wt. (kg)	Total body water (l)	Days	Amino acids (g N/d.)	PUN (mg/100 ml) Before	PUN (mg/100 ml) After	Plasma creatinine (mg/100 ml) Before	Plasma creatinine (mg/100 ml) After	App. nitrogen balance (g N/d.) Non-corrected	App. nitrogen balance (g N/d.) Corrected
3	74	31[a]	7	2.65	83	75	18	18	2.4	2.7
4	51	28[a]	7	2.65	68	66	12	11	1.5	1.6
13	75	46	7	2.65	59	59	17	16	1 7	1 7
14	58	32[a]	7	2.65	54	39	10	9	1.5	2.1
15	71	39[a]	7	2.65	57	55	17	17	1.9	2.0
17	52	27[a]	7	2.65	61	54	11	12	2.0	2.3
18	54	33[a]	4	2.65	57	56	14	14	1 7	1 7
			8	5.3	56	81	14	13	4.1	3.1
			4	2.65	81	77	13	14	1.3	1.6

Determined by tritiated water

Table V *Results from the series D patients treated with the eight essential amino acids and both histidine and arginine*

Case no	B. wt. (kg)	Total body water (l)	Days	Amino acids (g N/d.)	PUN (mg/100 ml) Before	After	Plasma creatinine (mg/100 ml) Before	After	App. nitrogen balance (g N/d.) Non-corrected	Corrected
(a) Series D1 Daily infusion of essential amino acids										
5	58	32[a]	4	3.8	51	51	11	11	2.2	2.2
			4	3.8	54	44	11	11	2.3	3.1
8	67	40	5	3.8	71	62	1	11	1.4	2.1
9	62	33[a]	4	3.8	84	75	14	14	1.6	[illegible]3
10	59	33[a]	5	3.8	34	39	7	7	3.7	3.4
11	49	27	7	3.8	69	50	13	12	1.9	2.6
16	54	30[a]	17	3.8	115	59	10	9	1.8	2.8
			13	3.8	83	49	12	11	1.5	2.2
17	49	27[a]	6	3.8	76	57	12	12	2.8	3.7
19	56	31	14	3.8	140	90	17	15	0.8	1.9
(b) Series D2. Infusion every second day of essential amino acids										
5	58	35[a]	18	7.4	88	39	12	10	1.5	2.4
6	45	25	10	7.4	114	78	10	10	0.9	1.8
	45	25	6	7.4	99	81	12	13	1.8	2.6
7	56	31	16	7.4	115	76	11	11	2.5	3.2
8	67	40	6	7.4	75	57	11	9	0.2	1.4
12	56	31	6	7.4	94	55	10	9	3.0	5.0

[a] Determined by tritiated water

spite of severe complications. In two of them the nitrogen balance was determined also during one or more periods before the complication occurred (case 1 Tables II and III, case 5 Tables II and V). The pre-complication periods of case 20 are not recorded in the Tables because she was treated alternately with I. and oral amino acid supply. In this group the balance figures are not reliable throughout, since these patients did not always consume their entire food ration and sometimes vomited. These losses were not considered when the balances were calculated.

Effect of the pertinent amino acid infusions on release of insulin and arginine

In separate investigations on severely uraemic patients (not included in the Tables) we studied the influence of arginine and histidine on the serum levels of insulin, HGH and blood glucose. In two patients these amino acids were infused I. together with the essential ones in the same dose as in series D. HGH and insulin were determined in serum samples obtained just before and 1 hour after the start of an infusion. A third sample was taken after 4 hours, at the time when the infusion was

Table VI. *Effect of intercurrent infection during treatment*

EAA = essential amino acid, EAA + H = essential amino acid + histidine

Case no.	B. wt. (kg)	Total body water (l)	Days	Type of infusion	Amino acids (g N/d.)	PUN (mg/100 ml) Before	After	Plasma creatinine (mg/100 ml) Before	After	App. nitrogen balance (g N/d.) Non-corrected	Corrected
1	75	45	5	EAA	2.2	120	141	17	16	0.9	−1.0
5	60	33[a]	14	EAA	2.[illegible]	108	85	15	17	−0.2	+0.3
			[illegible]0	EAA + H	[illegible].65	85	93	17	16	1.1	1.0
			7	0	—	93	70	18	16	−1.2	−0.1
			5	EAA + H	1.33	70	72	16	16	0.7	0.6
20	54	30	11	EAA + H	2.65	55	80	13	14	1.0	−0.[illegible]

[a] Determined by tritiated water

Diagnosis of the infection: case 1 thrombophlebitis with staphylococcal infection and sepsis, case 5, sinusitis with bronchitis; case 20, pulmonary tuberculosis.

completed. Blood glucose level was measured simultaneously. The patients were fasting 12 hours prior to and during the infusion.

A third uraemic patient was kept on nitrogen-free calorie-rich carbohydrate diet (Hycal® Beecham, Brentford, England). After an initial period on this diet only treatment with i. v. infusions of essential amino acids with or without histidine and/or arginine was started in alternate periods. The first serum samples for HGH and insulin determination were taken after 10 days on essential amino acids + histidine in doses as in series C. Further samples were taken after 3 days with infusions of essential amino acids + histidine and arginine in doses as in series D 1. After another 2 days, with essential amino acids only a new sample was taken. Two more samples were taken when essential amino acids + arginine but no histidine were administered.

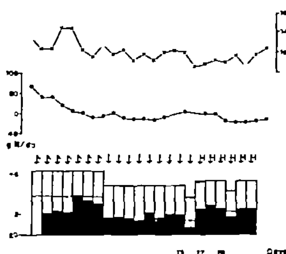


Fig. 1 (case 17). A 22-year-old woman with chronic glomerulonephritis and uraemia. White bars, g N administered (amino acids + food); black bars, apparent nitrogen balance g/day; corrected apparent nitrogen balance; ● PUN concentration (mg/100 ml); plasma creatinine concentration (mg/100 ml); Infusion of the 8 essential amino acids with both histidine and arginine; infusion of only the 8 essential amino acids; infusion of the 8 essential amino acids with histidine.

RESULTS

Series A nitrogen-poor diet without amino acid supply (Table II)

All patients except three were in a negative apparent nitrogen balance during this period of quite insufficient supply of nitrogen even when corrections for changes in total body urea had been made. The mean corrected balance of 18 periods from 15 patients was -0.91 ± 0.21 g N/day. The plasma urea decreased during these periods in all cases, whereas the plasma creatinine level remained essentially unchanged.

Series B nitrogen-poor diet supplemented with the eight essential amino acids infused daily (Table III)

In all patients a positive apparent nitrogen balance was obtained. The mean corrected balance of the three subjects receiving 2.2 g nitrogen was 1.60 g. The plasma urea decreased in four patients and increased slightly in one.

Series C nitrogen-poor diet supplemented with the eight essential amino acids and histidine infused daily (Table IV)

The corrected balance was positive in all cases. The mean corrected balance when 2.6 g nitrogen was infused daily was 1.96 ± 0.14 g N/day. PUN decreased in all cases except when double the dose of amino acids was given to case 18. In this period his plasma urea nitrogen increased and he became nauseated and vomited. When changing to the lower dose of amino acids these symptoms disappeared. In patient 4 the urea decrease was slight.

Series D nitrogen-poor diet supplemented with the eight essential amino acids and arginine and histidine infused daily or every second day (Table V)

The mean corrected balances were 2.63 ± 0.19 (D 1) and 2.73 ± 0.52 (D 2) g nitrogen, respectively. In all cases (except cases 5 and 10 in series D 1) a decrease in PUN was recorded.

Series E effect of intercurrent infection during the treatment (Table VI)

In all three studies the corrected balance was considerably less positive than when the same cases were studied before the complication. Because the protein intake in this group may sometimes have been lower than the figure used for calculation, the balance values may erroneously have turned out too high. The PUN increased markedly in two cases (cases 1 and 20) and remained essentially unchanged in the third (case 5) during the development of the complications.

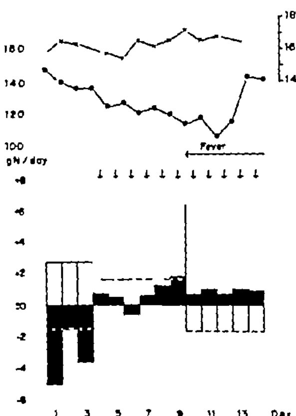


Fig. 2 (case 1). A 51-year-old man with polycystic kidneys and uraemia. On the first three days of the study the patient kept the nitrogen-poor diet but no amino acids [illegible]ere administered. On day 9 an infectious thrombo- [illegible] [illegible]th fever complicated the case. Symbols as in [illegible] 1

In order to illustrate the general course of the experiments two cases are graphically presented. Case 17 (Fig. 1) included in series B C and D was treated with all three amino acid regimens during consecutive periods. In this case the corrected balance was more positive when arginine and histidine were given together with the essential amino acids than if arginine was omitted. During the histidine period the balance was more positive than when the essential amino acids were given alone without histidine.

During the first weeks of amino acid administration to case 1 (Fig. 2) included in series B and E, the corrected nitrogen balance was strongly positive. However when he developed staphylococcal infection with thrombophlebitis and fever the balance at once became negative and the plasma urea concentration increased markedly.

Study of the effect of the amino acid infusions on the production of insulin and HGH

This revealed that the serum concentration of these hormones and the blood glucose concentration remained within normal ranges during and after infusions of the pertinent essential amino acid solutions with or without additions of histidine and/or arginine to the three uraemic patients studied in this respect.

DISCUSSION

Intravenous nutrition with amino acids has proved to be of value for patients in the post-traumatic state by reducing the net protein catabolism. However the solutions used (protein hydrolysates or mixtures of crystalline amino acids) contained considerable amounts of non-essential amino acids. Such solutions are thus unsuitable for uraemic patients since they tend to produce an excess of urea and to prevent the utilization of the urea pool for protein synthesis.

Rose and Dekker (29) demonstrated that protein-depleted rats can use urea as a source of non-essential nitrogen in protein synthesis. Giordano (16) showed that uraemic patients can use their own urea for the same purpose when a synthetic diet was supplied with essential amino acids by mouth. It is now agreed that the utilization of urea for amino acid synthesis occurs after hydrolysis by urease to ammonia, in the intestine.

Although the intake of nitrogen was known and the losses of nitrogen with the urine were accurately determined in our studies, the nitrogen balances could not be correctly calculated since the losses of nitrogen in sweat and stools were not directly determined. Consequently these losses could not be included and we had to confine ourselves to calculation of the "apparent nitrogen balance".

In a separate study we found the mean daily faecal nitrogen obtained from four uraemic patients on the standard nitrogen-poor diet (examined during 11 to 49 days, in total 88 days) to be 0.89 g/day (range 0.73–1.05). The faecal nitrogen excretion was independent of whether the amino acids were infused or not. Our mean value does not differ from the mean of 0.86 g nitrogen/day found in the faeces of healthy subjects receiving our diet of 2.7 g nitrogen/day (31).

The published values for the nitrogen loss by the skin are conflicting. The nitrogen loss from the skin has been found to be 0.25–0.50 g/day in adult healthy man (7). Adopting the figure of 10 mg/kg b.wt./day suggested by Munro (26), our patients would have lost about 0.6 g nitrogen by this route. Thus a rough estimate of the extrarenal nitrogen loss in our patients would be 1.5 g/day.

On the nitrogen-poor diet before commencement of the amino acid therapy (series A, Table II) the "corrected apparent nitrogen balance" was negative in most cases. If the extrarenal losses are taken into consideration, the balance was markedly negative in all the patients. This was to be expected since the quantity of nitrogen supplied with the food was below 3.5 g/day considered to be the minimum for balanced nitrogen metabolism in healthy adults (4, 20). There was a pronounced decrease of plasma urea concentration in all cases during this period.

On the other hand, in all the five patients who received only the eight essential amino acids in addition to the nitrogen-poor diet (series B, Table III) the corrected balance became equilibrated even when the estimated extrarenal nitrogen losses were deducted. Concomitantly the plasma urea concentration remained stable or continued to decrease. This result indicates that the amino acids, when infused i.v., enhance protein anabolism also in severe uraemia, without increasing the urea pool.

The seven patients who in addition to the essential amino acids received histidine (series C, Table IV) had a "corrected apparent nitrogen balance" which was more positive than that of the former group. The differences in nitrogen balances obtained upon administration of essential amino acids with and without histidine are especially evident in patients 3 and 17 who received both types of treatment, with histidine supplementation being applied later. In both these patients the balance was more positive when histidine was added. The increase of the "corrected nitrogen balance" exceeded the amount of nitrogen supplied as histidine.

Previously published results (8) indicate that histidine is of importance for the nitrogen balance and also for the Hb synthesis in uraemic patients.

A more clearcut demonstration of the special effect of histidine on the nitrogen balance in uraemia has previously been obtained by a study of a uraemic patient on a nitrogen-free carbohydrate diet (1). In alternating periods the eight essential amino acids were administered either without or together with histidine or proline. Histidine and proline were given in isonitrogenous quantities. When histidine was administered the corrected nitrogen balance improved more than could be accounted for by the extra nitrogen supplied as histidine. Proline, on the other hand, improved the corrected nitrogen balance to an extent that was lower than the amount of nitrogen added as proline.

After administration of a non-essential source of ^{15}N to a healthy subject and to a patient in post-traumatic catabolism, we recovered ^{15}N incorporated in histidine isolated from muscle and plasma protein (14). In contrast to this result no labelled histidine was found in the corresponding proteins from uraemic patients (10), indicating that histidine behaves like an essential amino acid in uraemia.

In the present investigation there was only a moderate difference in nitrogen balance between patients receiving essential amino acids with or without histidine. One reason for this may be that the nitrogen-poor diet contained histidine.

Histidine is reported by many authors to be essential to most mammals except man. However, it is also essential to newborn infants (22). Thus amino acid synthesis seems to be more advanced in this respect in man than in other mammals. Uraemia or uraemic toxicity seems to deprive man of this "superiority".

The group of 11 patients who received both arginine and histidine together with the essential amino acids (in 16 studies) exhibited the most positive corrected balance of all the groups. In all but two of the cases the plasma urea concentration decreased simultaneously with the increase of the nitrogen balance. Two of the patients of this group (cases 16 and 17) were also tested with essential amino acids only (series B) and case 17 also with histidine without arginine (series C). The high nitrogen balance caused by arginine (in the presence of histidine) was quite obvious when the results of different balance periods in these patients were compared. However, the increase of the nitrogen balance when arginine was supplied was lower than the additional nitrogen from the arginine.

It is well known that arginine may stimulate production of insulin and of growth hormone in man (27). However it does not seem likely that the increase of the nitrogen balance produced by addition of arginine (corresponding to 1 12 g N) was caused by such an effect, since no increases of these hormones were observed in the serum of the three patients investigated in this respect. In another study (2) a uraemic patient on a nitrogen-free carbohydrate diet was treated with amino acid infusions. Nitrogen added in the form of arginine was utilized only when histidine was given simultaneously

Some of the substances incriminated as toxic in uraemia are suspected of being closely related to the urea cycle in which arginine is an important step. These metabolites include methylurea, methylguanidine and guanidino-succinic acid (17 18 30) Although the metabolic pathways are unknown it has been assumed that arginine could play a role as a precursor to the toxic metabolites (6). However we have never observed any untoward effect of i.v arginine supply for periods up to 1 month to uraemic patients.

Numerous papers report that a diet containing restricted amounts of protein with a high biological value has a beneficial effect on the nitrogen metabolism in uraemia. Referring to several other investigations and to their own experience, et al (9) recently concluded that the food uraemic patients should contain about 0.5 g animal protein kg b.wt./day in order to maintain a balanced nitrogen metabolism. This quantity corresponds to a daily ration of about 5 g nitrogen/day for a patient weighing 60 kg. As our standard diet contains only 2.2–3.0 g nitrogen/day it is not surprising that the nitrogen balance was strongly negative as long as the patients did not receive any additional nitrogen.

The subjects in our series B (the essential amino acids i.v) received about 2.2 g nitrogen by infusion, making up their total nitrogen supply to nearly the suggested minimum requirement resulting in an improved nitrogen balance. The subjects of groups C and D I received more than 5 g nitrogen/day and without exception their nitrogen balance became positive However it should be observed that the plasma urea concentration decreased in most of our patients, whereas this concentration increased in the patients of Ford et al. to whom all the nitrogen was provided by mouth as protein. The reason for this difference is most probably that our patients received a much larger part (about 60%) of their nitrogen as essential amino acids, thereby inducing a consumption of urea for protein synthesis, whereas the large part of non-essential nitrogen in the food protein in the study of Ford et al. brought about an increased urea production.

The three cases in series E demonstrate that intercurrent infections may interfere with the nitrogen metabolism. We have also observed that factors such as insufficient caloric intake, lack of non-essential nitrogen, potassium depletion corticosteroid therapy or cardiac insufficiency have resulted in a deterioration of the nitrogen balance *and an increase in plasma urea concentration.*

The i.v amino acid therapy has been used in this hospital for about three years, also on a considerable number of uraemic patients, on whom the nitrogen balance was not studied.

Our routine procedure is now daily infusions of the solution containing the essential amino acids and histidine. The amino acids are instituted after detoxification of the patient by a few days on the nitrogen-poor diet only or by a single peritoneal dialysis. A prerequisite for the success of the treatment is that disturbances in water electrolyte and acid-base metabolism are corrected and complicating factors such as infections and others mentioned above are eliminated as far as possible before the amino acids are administered.

ACKNOWLEDGEMENTS

This investigation was supported by grants B69-19X-573-04, B69-61P 2618-01 and B70-19X-1002-05B from the Swedish Medical Research Council and by grants from the foundation "Therese och Johan Anderssons Minne and the Semper Company research allowances.

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Congress Announcements

Le Congrès Annuel de La Société de Broncho-oesophagologie et de Gastroscopie de langue Française se déroulera à Stresa, Italie, Lac Majeur au Palais des Congrès, sous la Présidence d'honneur des Professeurs A. Soulas et P Mounier Kuhn et du Président de tour Prof G Merlo du 12 au 13 Mai 1972.

Secrétariat Général du Congrès. Prof Dott. G Merlo Baluardo Lamarmora, 2 18100 Novara, Italie.

Sujets des deux tables rondes: Les gastrites, L'interet de la fibroscopie dans l'investigation bronchique et oesophagienne. Suivront communications libres.

The IXth International Congress of Nutrition will be held in Mexico City September 3–9 1972. In the scientific program, composed of over 40 symposia or panel sessions and near 600 short communications, considerable attention is given to the practical aspects of the nutritional sciences. An interesting social program free of cost for participants has been designed. Registration fee: US$ 55 00 for full members and US$25 00 for accompanying members.

Information and registration. IX Congreso Internacional de Nutrición, P O Box 22 112, México D.F México

The 17th Annual Clinical Conference "Neoplasms of the Head and Neck" will be held in Shamrock Hilton Hotel, Houston Texas, USA, November 2–3 1972.

The 26th Annual Symposium on Fundamental Cancer Research, Immunological Aspects of Neoplasia will be held in Shamrock Hilton Hotel, Houston Texas, USA, March 6–9 1973.

Acta med. scand. Vol. 191 pp. 369–376, 1972

PLASMA CORTICOSTEROIDS IN PRIMARY AND SECONDARY ADRENOCORTICAL INSUFFICIENCY

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Abstract. Plasma corticosteroids have been studied in primary (p.a.i.) and secondary adrenocortical insufficiency (s.a.i.). In eight cases of p.a.i. and in four cases of s.a.i. the circadian rhythm was absent; all except one had low values of plasma corticosteroids at 7 a.m. The 4-hour intravenous ACTH test was carried out in nine cases of p.a.i. and in eight cases of s.a.i. In p.a.i. no adrenal response was found; in s.a.i. no response was found in one case, subnormal responses in six cases and normal response in one case. Baseline values of radioimmunologically determined serum corticotropin were elevated in three cases of p.a.i., in two cases of p.a.i. circadian rhythm of serum corticotropin was demonstrated. Insulin tolerance test (ITT) was carried out in eight cases of s.a.i., no pituitary-adrenal response was found. The diagnostic values of ITT and the metyrapone test in s.a.i. are discussed. It is concluded that early morning baseline values of plasma corticosteroids are often abnormally low in p.a.i. and in s.a.i. During 4-hour ACTH test no adrenal response is to be found in p.a.i., in s.a.i. variable response will be found. A partial response is diagnostic for s.a.i. Subnormal responses during metyrapone test but normal during ITT have been reported in few cases of s.a.i. However, subnormal responses during ITT indicate that the responses during the metyrapone test are also subnormal.

In primary chronic adrenocortical insufficiency Morbus Addisonii (p.a.i.) (1) destructiv lesions affect all three zones in the adrenal cortex and compromise the secretion of cortisol, corticosterone and aldosterone. However in clinically manifest adrenocortical insufficiency there may be a remnant adrenal function, as the urinary excretion of 17-hydrocorticosteroids (17-OHCS) (19 30) and of 17-ketogenic steroids (17-KGS) (8, 13) is often normal or only moderately reduced.

In secondary adrenocortical insufficiency (s.a.i.) there is an adrenal atrophy secondary to a diminished secretion of adrenocorticotropin (ACTH) in the anterior pituitary. As the production of aldosterone in zona glomerulosa is extensively independent of the ACTH production (10 14) it is mainly the production of cortisol and corticosterone that is compromised. In clinically manifest s.a.i. the urinary excretion of 17-OHCS and 17-KGS is mostly reduced, but in a few cases a low normal urinary excretion may be found (8 13 31 42).

Thus the diagnosis adrenocortical insufficiency may not always be established from the basal urinary excretion of corticosteroids. On the other hand estimations of the urinary corticosteroid excretion during stimulation with exogenous ACTH give more precise information on the secretory capacity of the adrenal cortex in p.a.i. and s.a.i. On ACTH stimulation daily for 2–3 days the urinary excretions of 17-OHCS (19 24) and of 17 KGS (8 13) do not increase in Addison's disease, while in s.a.i. a certain but subnormal increase in 17-OHCS (24 30) and 17-KGS (8, 13) is seen in the urine. The *actual* secretory capacity of the adrenal cortex can thus be evaluated by a short-term ACTH test, whereas the *potential* secretory capacity can only be evaluated by a several-day ACTH test.

In six patients with clinically and biochemically verified panhypopituitarism Chakmakjian et al. (8) did not find any definite increase in 17-KGS during daily ACTH stimulation for five days. Thus the 2 or 3-day ACTH test, usually used in the clinic, is not absolutely reliable for differentiating between p.a.i. and s.a.i.

In contrast to s.a.i., blood ACTH levels are elevated in untreated Morbus Addisonii (4 17). Determinations of blood ACTH, therefore, would be the method of choice in distinguishing between

Table I. *Clinical and biochemical data on nine p.a.i. patients*

Case no.	Sex	Year of diagnosis	Addison crises	Mucosal and skin pigmentation	BP (syst./diast.) (mmHg)	TB	Serum Na low serum K high	Year of present study	Age (y)	Height (cm)	B.wt. (kg)	17 KGS (mg/24 h) Baseline values	17 KGS (mg/24 h) Day of 4-hour ACTH test
1	♀	1965	No	Yes	105/70–95/75	N	No	1965	47	166	50	8.0–11.3	10.5
2	♀	1952	Yes	Yes	95/75	Pulm.	Yes	1965	57	162	60	6.1	2.6
3	♂	1954	Yes	Yes	70/	Pulm. urog.	Yes	1967	64	174	74	5.4	3.6
4	♀	1968	Yes	Yes	80/60	Pulm.	Yes	1968	63	155	57	9.2	3.3
5	♂	1956	Yes	Yes	—	Urog.	Yes	1965	47	176	84	5.9–8.0	6.5
6	♂	1962	No	Yes	105/80–110/80	No	No	1968	24	175	56	8.2	4.6
7	♀	1963	Yes	Yes	80/70–85/65	No	Yes	1968	29	170	75	—	0.1
8[a]	♀	1949	Yes	Yes	90/60	Pulm.	Yes	1970	71	163	72	4.1	4.0
9[a]	♀	1950	Yes	Yes	100/70–95/60	No	Yes	1970	75	163	69	3.4	2.5

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these conditions. A method for such determinations is not yet available for routine diagnostic investigations. However by the metyrapone test, introduced by Liddle et al. (33) the ACTH secretion capacity can be evaluated indirectly by alterations in urinary excretions of corticosteroid metabolites (20, 21, 33) or from alterations in plasma 11-deoxycortisol (Comp. S) (7). To some extent the insulin tolerance test (ITT) introduced by Bliss et al. (5) can be compared with the metyrapone test. The response of plasma corticosteroids during ITT is an indirect expression for the release of ACTH (2, 4).

An evaluation of the hypothalamo-pituitary adrenal activity from determinations of plasma corticosteroids has been of increasing interest in recent years. The diagnostic value of fluorimetric determination of plasma corticosteroids (41) during basal conditions (circadian rhythm) and during 4-hour ACTH test in p.a.i. and s.a.i. and of ITT in s.a.i. is investigated in the present study. Furthermore, serum corticotropin is determined in Addison's disease. Steroid-induced adrenocortical insufficiency has not been investigated and is not discussed.

MATERIAL

Primary chronic adrenocortical insufficiency

The material comprised nine patients with Addison disease. Table I surveys some clinical and biochemical data. Only in cases 3, 5 and 8 were there roentgenologically demonstrable calcifications at the site of the adrenals. Sputum and urine cultures revealed tuberculosis pulmonalis in cases _, 3, 4 and 8 and tuberculosis urogenitalis in cases 3 and 5. Adrenalitis tuberculosa is thus probable in cases 3 and 5 and possible in cases 2, 4 and 8. Investigation for adrenocortical antibodies in the blood (40) in four patients (nos. 1, 6, 8 and 9) gave a positive reaction in case 1 and a weakly positive reaction in case 6.

Urinary 17-KGS/24 h was determined during basal conditions and during a 4-hour ACTH test (see below) without substitution treatment. Compared with the control material of Jørgensen (27) the baseline values of urinary 17-KGS in Table I were all normal. During the ACTH test no increased excretion of the 4-hour urinary 17-KGS was seen. In 13 normal individuals, except one whose urine samples were by mistake mixed up, Asfeldt and Nielsen (3) found an increase in 17-KGS of 4.9–4.3 mg/24 h rise during this test.

A several-day ACTH test had previously been carried out only in one patient (no. 6), in whom no increased urinary excretion of 17-KGS was seen during i.m. infusion of 25 IU ACTH per day for two days.

The diagnosis Morbus Addisonii is thus based on clinical findings and on the observation of an insufficient response in urinary 17-KGS during short-term ACTH test.

Secondary adrenocortical insufficiency

The material comprised 12 patients. There were no clinical signs of Morbus Addisonii. The first six patients in Table II had been operated on for craniopharyngeoma 9 years before the investigation. Since the operation patients 11, 12, 13 and 15 had received cortisone and thyroidin. In patient 12 stalk section was undertaken two years before the investigation, subsequently he received cortisone thyroidin and testosterone.

CASE REPORTS

Case 17

Female born in 1944. She had primary amenorrhea and was sex chromatin positive. Because of reduced thyroid and gonad function, found in 1961 she received thyroidin

Table II. *Clinical and biochemical information on 12 patients with hypopituitarism and s.a.i.*

Case no.	Sex	Age (y.)	Height (cm)	B.wt. (kg)	Urinary gonadotropin (m.u./24 h)[a]	Urinary 17-KS (mg/24 h)	Reduced thyroid function	Plasma STH response during ITT	17 KGS (mg/24 h) Baseline value Total	Per m² BSA	During 4-hour ACTH test
11	♂	21.2	159	47.8	<3	2.8	Yes	No	0.0	0.0	—
12	♂	19.0	161	59.6	<3	0.7	Yes	No	0.0	0.0	0.0
13	♂	11.0	134	40.5	<4	1.8	Yes	No	1.5	1.2	—
14	♂	14.2	133	32.4	<3	0.0–0.8	Yes	No	0.0	0.0	0.0
15	♂	56.3	175	91.0	<4	1.0	Yes	—	1.4	0.7	—
16	♀	12.5	131	32.5	<3	0.1	Yes	—	0.7	0.6	0.3
17	♀	22.8	180	73.5	<3	1.4	Yes	—	3.6–5.5	1.9–2.8	3.1
18	♀	29.6	149	39.9	<4	3.3	No	—	2.3	1.8	1.9
19	♂	15.0	147	40.2	<3	0.9	No	No	0.0	0.0	—
20	♂	19.0	142	32.8	<3	0.6	No	No	0.3–1.0	0.3–0.9	2.2
21	♂	28.5	168	65.1	<3	2.7	Yes	No	2.3	1.3	5.4
22	♂	36.5	178	68.3	<3	1.7	Yes	—	0.0	0.0	—

[a] m.u. = mouse units.

and estrogens. Subnormal response during metyrapone test (7) was found in 1963 (plasma Comp. S 2.0–4.6 μg/100 ml) and in 1970 (plasma Comp. S 0.3–1.6 μg/100 ml, plasma cortisol 2.9–0.1 μg/100 ml). Sella turcica was normal.

Case 18

Female, born in 1939. After prolonged delivery in 1967 she developed Sheehan's syndrome with amenorrhea, failure to lactate and later decrease of axillary and pubic hair. Circulatory failure in relation to septicemia (1968) responded well to cortisone. 17-KGS and 17-ketosteroids (17-KS) were 0.5 and 0.8 mg/24 h urine respectively. Sella turcica was normal.

Case 19

Male, born in 1952. In 1966 he was operated on for hydrocephalus internus. In 1967 diabetes insipidus and adrenocortical insufficiency (17-KGS 0.6 mg/24 h urine) was diagnosed. Sella turcica was normal.

Case 20

Male, born in 1947. From the age of six years growth was retarded. In 1966 the bone age was 12 years. He was psychoinfantile and had no development of secondary sex characteristics. Sella turcica was normal.

Case 21

Male, born in 1940. In 1950 retarded growth was recognized. In 1955 thyroidal ¹³¹I uptake was decreased; bone age was 12 years. In the last six years he has not grown in height. Since 1955 he received thyreoidin and since 1965 androgens.

Estimated from serum-protein-bound iodine reduced thyroid function was found in patients 17 and 21 and in the operated patients just after the operation. Patients 13, 16, 19 and 20 had retarded height growth. Patients 11, 1., 13, 14 and 19 had manifest diabetes insipidus; postoperatively patients 15 and 22 had diabetes insipidus that later vanished.

Radioimmunological determination of somatotropin (STH) in plasma was carried out in seven patients during ITT. An increase of at least 5 ng/ml during ITT was evaluated as normal response (22).

In individuals with normal somatic development urinary 17-KGS are positively correlated to the body surface (44). In Jørgensen (27) control material for urinary 17-KGS regard has only been paid to age variations, and as some of the patients in Table II are growth-retarded the baseline values of 17-KGS in these patients cannot as a matter of course be compared with Jørgensen control values. The baseline values in Table II, determined in 24 hours without cortisone treatment, are evaluated in relation to value of 17-OHCS in the urine of 3.1 ± 1.1 mg m² BSA/24 h that was calculated by Migeon et al. (37) in normal individuals aged between 4 months and 76 years. Baseline values of 17-KGS lower than 2.0 mg m² BSA/24 h are regarded as abnormally low. However, determinations in the same urine will give somewhat higher values of 17-KGS than of 17-OHCS, but this source of error is accepted, as values of urinary 17-OHCS will be lower than the baseline values of urinary 17-KGS given in Table II. In cases 12, 14, 16, 18, 20 and 21 subnormal responses were found in urinary 17-KGS during 4-hour ACTH test.

The diagnosis of s.a.i. must be regarded as definite for the patients in Table II, even though the diagnosis is not further substantiated by the metyrapone test (except pat. 17) and by an ACTH test lasting several days. They all presented several clinical and biochemical signs of pituitary insufficiency: baseline values of 17-KGS were abnormally low in all cases except patient 17, in whom s.a.i. was confirmed by the metyrapone test.

Table III. *Circadian rhythm of plasma corticosteroids in p.a.i. and s.a.i.*
Controls published by Asfeldt (2)

Case no	Plasma corticosteroids (μg/100 ml) 7 a.m.	9 a.m.	12 a.m.	07 p.m.	10 p.m.	Year
p.a.i.						
1	13.	14	15	15	9.2	1965
1	2.0	—	1.2	1.0	1.5	1967
2	4.5	4.7	5.3	5.0	5.3	1965
3	3.8	3.7	3.8	3.3	3.3	1967
4	10.[illegible]	11.0	8.3	10.0	8.0	1968
5	6.6	6.2	6.5	4.[illegible]	4.5	1965
6	5.4	5.8	5.6	5.8	5.8	1968
8	5.2	5.8	6.4	5.9	4.8	1970
9	4.0	3.7	4.0	3.5	2.9	1970
s.a.i.						
15	1.3	[illegible].3	2.9	2.9	2.9	1965
22	1.1	1.4	1.3	1.8	2.4	1967
17	6.0	—	10.5	3.5	4.8	1967
17	3.3	4.0	5.0	3.3	2.7	1970
18	[illegible].2	—	—	0.5	0.6	1969
Controls (mean ± 2 S.D.)						
Female (n = 10)	13.4–33.5	6.3–19.9	6.7–16.9	4.5–13.7	1.1–11.5	
Male (n = 8)	8.5–26.7					

METHODS

Heparinized venous blood samples were taken at 7 a.m., 9 a.m., 1 p.m., 7 p.m. and 10 p.m. for determinations of plasma corticosteroids (circadian rhythm). In three patients (nos. 2, 3 and 5) one or more venous blood samples were taken at the same time for radioimmunological determinations of serum corticotropin.

The ACTH test was carried out as already described (2): 250 μg β^{1-24}corticotropin, dissolved in 500 ml 0.9% NaCl, was infused from 9 a.m. to 1 p.m. Before and during the ACTH test, at intervals of one hour heparinized blood samples were drawn for determination of plasma corticosteroids.

Table IV. *Serum corticotropin and plasma corticosteroids during basic conditions in 3 patients with Addison's disease not receiving cortisone*

		Serum corticotropin (pg/ml)	Plasma corticosteroids (μg/100 ml)
Case 5 1967	8 a.m.	1000	8.1
	12 a.m.	420	8.6
	07 p.m.	400	7.9
	10 p.m.	380	9.2
Case 3 1967	8 a.m.	585	3.7
	10 p.m.	364	3.3
Case 2 1965	8 a.m.	>1000	4.3
Normal subjects (n = 106)	8 a.m.	50–200 (16)	

ITT was carried out as earlier described (2). An i.v. injection of crystalline insulin 0.10 IU/kg b.wt. was given; at each subsequent 10th min heparinized venous blood samples were taken for determination of plasma corticosteroids and plasma STH. At the same time capillary ear blood was drawn for blood sugar determinations. During the 24 hours of the ACTH test, ITT and circadian rhythm no cortisone treatment was given.

Plasma corticosteroids were determined fluorometrically (41). In this way 11-OHCS (cortisol + corticosterone) are determined. With a standard deviation in replicate determinations of 0.32 μg/100 ml, the lower detection limit of changes in plasma corticosteroids is found to be 1.6 μg/100 ml ($p \sim 0.001$).

Urinary 17-KGS and 17-KS were determined by Statens Seruminstitut (45) or by Medicinsk laboratorium (15, 36).

The radioimmunological determination of serum corticotropin was carried out by Dr An. Gabkov, Medicinsk fysiologisk institut B, Copenhagen. The accuracy of the analysis, given by the variance, was 7.4% for all concentrations (16).

The radioimmunological determination of plasma STH was carried out by Dr I. Høyer from this hospital.

Blood sugar was determined by the method of Hagedorn et al. (18).

RESULTS

The circadian rhythm of plasma corticosteroids and the values of the serum corticotropin are

Table V *Plasma corticosteroids during 4-hour i.v. ACTH test in p.a.i. and s.a.i.*

Controls published by Adeldt (2)

Case no.	Plasma corticosteroids (µg/100 ml) 9 a.m.	10 a.m.	11 a.m.	12 a.m.	01 p.m.	02 p.m.	Year
p.a.i.							
1	14.	15.	14	13.	14	14	1965
2	3.1	3.4	3.3	3.1	3.1	3.4	1965
3	3.1	3.1	3.4	3.3	3.1	3.3	1967
4	9.0	9.1	8.3	8.5	8.9	8.9	1968
5	4.9	4.8	5.2	4.8	4.3	4.8	1965
6	5.1	4.2	4.2	3.9	3.5	3.5	1968
7	1.1	—	—	—	0.9	0.7	1968
8	6.4	6.4	5.9	5.3	5.0	—	1970
9	3.5	3.8	2.9	2.9	2.6	—	1970
s.a.i.							
11	2.0	3.7	2.5	2.5	2.9	2.2	1966
12	1.9	—	—	—	4.0	—	1967
14	3.1	—	—	—	6.9	6.3	1967
16	2.8	—	—	—	5.0	—	1966
17	4.7	20.5	24.3	27.1	28.5	—	1967
18	0.6	2.9	3.1	3.1	3.6	3.5	1969
20	2.7	—	—	—	20.4	19.1	1966
21	5.9	—	—	—	22.4	—	1969
Controls (mean ± 2 S.D.)							
Female (= 10)	8.–24.4	22.5–33.7	25.8–41.9	28.8–46.9	29.8–53.4	32.3–56.7	
Male (= 8)	8.2–22.4	21.5–29.4	27.5–32.9	28.5–36.3	32.4–41.1	30.4–41.7	

shown in Tables III and IV. In 1965, before cortisone treatment was introduced, normal baseline values of plasma corticosteroids were found in patient 1. However, after two year's cortisone treatment baseline values of plasma corticosteroids, except the 10 p.m. value, were abnormally low. In the other patients in Table III the plasma corticosteroids were abnormally low at 7 a.m. In the course of the day, however, normal values of plasma corticosteroids were found with increasing frequency. With the exception of patients 1 and 4 the values of plasma corticosteroids remained at almost the same level during the whole 24 hours.

All values of serum corticotropin were elevated in p.a.i. (Table IV). In cases 3 and 5 a clear fall in serum corticotropin is seen in the course of the day, but plasma corticosteroids did not alter and were even normal in case 5.

During the 4-hour ACTH test (Table V) no increase in plasma corticosteroids was seen in p.a.i. On the other hand highly variable responses were found in s.a.i. In case 11 no significant response was found, in six cases significant but subnormal responses were observed, in case 17 plasma corticosteroids increased to a normal level at 1 p.m.

During ITT the fall in blood sugar was 34–58 % which corresponds with that found in normal individuals (2).

In normal individuals a significant increase in plasma corticosteroids is seen 40 min after the injection of insulin, and maximum values are seen at 50–60 min. The minimum limit of maximal increase in plasma corticosteroids in normal individuals is taken to be 6 µg/100 ml (2).

All patients in Table VI had a subnormal response in plasma corticosteroids during ITT.

DISCUSSION

Earlier determinations of baseline values of plasma 17-OHCS (46) or of fluorimetrically determined plasma corticosteroids (34, 39) in adrenocortical insufficiency have been carried out on single blood samples, which have chiefly been taken in the morning. Both in p.a.i. (39, 46) and s.a.i. (34, 46) normal and subnormal baseline values were found.

In the present study, in contrast to the above-

Table VI *Blood sugar and plasma corticosteroids during ITT in s.a.i.*

Case no	Year	Blood sugar (mg/100 ml) 0'	10	20	30	40	50	60	Plasma corticosteroids (µg/100 ml) 0	10	20	30	40'	50	60'
11	1964	67	40	28	47	97	—	—	1.0	1.6	1.1	1.6	1.9	—	—
1	1967	76	47	32	30	64	—	—	3.8	2.7	2.7	2.5	2.9	—	—
13	1968	86	63	48	62	70	80	90	1.6	—	1.6	1.9	2.2	—	2.4
14	1967	82	74	52	38	47	—	—	1	—	2.4	2.7	2.7	—	—
17	1967	83	53	30	45	56	—	—	9.1	9.6	—	9.3	9.6	—	—
17	1970	74	56	37	43	39	67	71	3.3	4.6	3.9	4.3	4.8	4.1	4.1
19	1968	64	55	58	41	71	78	95	2.3	2.3	2.6	2.3	2.6	3.2	2.7
20	1966	83	62	29	34	86	—	—	3.1	3.1	2.5	2.9	3.1	—	—
21	1969	73	64	35	45	53	59	55	2.5	2.8	2.5	3.1	3.1	3.5	3.0

mentioned investigations, baseline values of plasma corticosteroids were determined several times during the day and night.

Reduced values at 7 a.m. were found in all patients except one (no. 1 in Table III) both in p.a.i. and s.a.i. Later in the day normal values were obtained with increasing frequency. The baseline values of urinary 17-KGS were without diagnostic value in Morbus Addisonii. In contrast, the plasma corticosteroids at 7 a.m. were reduced in eight of nine cases. It was characteristic that plasma corticosteroids were around the same level during the whole day in adrenocortical insufficiency. Only early morning determinations of plasma corticosteroids are thus of diagnostic significance in adrenocortical insufficiency.

In earlier studies no adrenocortical response was observed in p.a.i. during one (29, 35, 38) to eight hours (12) maximum ACTH stimulation.

In s.a.i. Christy et al. (9) found almost no or subnormal increases in plasma 17-OHCS in nine cases during a 4-hour ACTH test. During 1-hour ACTH test similar responses in fluorimetrically determined plasma corticosteroids have been described by others (11, 26, 38). Jenkins and Elkington (25) have shown that patients with a reduced ACTH secretion capacity and s.a.i. as verified by the metyrapone test, might have a normal increase in plasma 17-OHCS during ACTH test.

The present results of the 4-hour ACTH test agree with the above mentioned observations. In all cases of p.a.i. and in one case of s.a.i. no response was found significant, but subnormal, responses were observed in six cases of s.a.i., and a normal response in one patient with s.a.i. It is thus obvious that a partial response during a 4-hour ACTH test is seen *only* in s.a.i. a finding which is of diagnostic significance in s.a.i.

The elevated values of serum corticotropin (Table IV) confirm earlier results of plasma ACTH estimated by biological (17) or radioimmunological (4) methods in Addison's disease. In untreated Morbus Addisonii the feedback inhibition of ACTH release is reduced, consequently the ACTH release increases and serum corticotropin values rise, but as also shown earlier (17) the circadian rhythm of serum corticotropin was maintained in cases 3 and 5. This shows that circadian rhythm and the feedback mechanism are two independent variables.

The metyrapone test appears to be the most sensitive test for the evaluation of the ACTH secretion capacity (6, 25, 28, 33), but for this purpose ITT, due to its simplicity, has been the object of increasing attention. The reliability of ITT in comparison with the metyrapone test has been investigated in several studies in which both tests were carried out in patients with presumed or verified reduced ACTH secretion capacity and s.a.i. In four patients with verified s.a.i. Kaplan (28) found subnormal response during ITT and the metyrapone test. Furthermore nine patients with enlarged sella turcica (chromophobe adenoma 6, acromegaly 3) were investigated. In three of them there was no response during ITT and the metyrapone test but a normal response during an ACTH test. In two of them there was a normal response during ITT and ACTH test but a subnormal response during the metyrapone test. The three remaining patients had normal responses during all three tests. In twelve patients (hypo-

physectomy 6, hypophyseal tumour 2, pituitary post-partum necrosis 2, idiopathic defect of ACTH production 2) with subnormal response during the metyrapone test Landon et al. (31) found reduced or no response during ITT and ACTH test, and in five patients (hypophyseal tumour 3 hypothalamic dysfunction 2) with normal response during ACTH test, a subnormal response was found during the metyrapone test and ITT In four patients with a verified reduced ACTH production Laron et al. (32) found a subnormal response during the metyrapone test and ITT Jacobs and Nabarro (23) carried out the metyrapone test and ITT in patients with various types of hypophyseal disease. Of 21 patients with normal response of plasma 17-OHCS during ITT there were 18 with a normal, and three (suspected hypopituitarism 1 mid-brain tumour 1 hypothalamic tuberculoma 1) with a subnormal response during the metyrapone test. Eight patients had no response either during ITT or the metyrapone test.

In the present study no response was observed during ITT in patients with a.a.i. (Table VI). A subnormal response during the metyrapone test was observed in one (no 17) of these patients.

With subnormal response during ITT a subnormal response has thus always been found during the metyrapone test, but in a few cases with normal response during ITT subnormal response is observed during the metyrapone test (23 28). These facts presumably reflect the greater *sensitivity* of the metyrapone test compared with ITT However as a screening test ITT is well suited, with a lack of response during ITT the metyrapone test is not required.

From a *clinical* point of view it should be emphasized that patients with a subnormal response during the metyrapone test might well have an adequate adrenocortical response to stress stimuli such as fever (6, 25) and surgery (43) The response during ITT could possibly be a guide as to whether adrenocortical substitution therapy is called for in fever and surgery (31).

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DETERMINATION OF PLASMA RENIN ACTIVITY BY RADIO-IMMUNOASSAY FOR ANGIOTENSIN II

A Modification of the Method of Boucher et al.

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Abstract. A procedure for coupling a radio-immunoassay for angiotensin II to a method for plasma renin activity measurement has been described. The incubation product of the method is angiotensin I. This is converted to angiotensin II by a preparation of human plasma containing converting enzyme. In the measurement of angiotensin formed standard curve with angiotensin I converted to angiotensin II in the same way as the unknown sample is applied. In this way correction is made for incomplete conversion of angiotensin I to angiotensin II by the converting enzyme preparation.

A modification of the method of Boucher et al. (1) will be described which makes it possible to perform the final angiotensin measurement by radio-immunoassay

METHOD

Human plasma was obtained and incubated as described by Boucher et al. (1) and modified by Nielsen and Møller (4). The eluate from the Dowex resin columns was, after the usual washing with ethylalcohol, finally evaporated and the residue dissolved in 1 ml tris-HCl buffer (0.2 M pH 7.5, 1% human albumin). 20 μl of this solution was added to 30 μl tris-buffer and 60 μl of a converting enzyme preparation in the form of human plasma, which had undergone transitory acidification (pH 3.6, 25°C in 20 min) in order to reduce the angiotensinase activity sufficiently (5). The mixture was incubated at 37°C in 2 hours. The reaction was stopped by submersion into ice water 200 μl of solution of I^{125}-tagged angiotensin II (Ciba), produced and diluted as described by Poulsen (5), was added to each sample. The assay proceeded as described by Poulsen.

A standard curve was obtained as follows: solutions of angiotensin I (Bio-Schwartz) dissolved in tris-buffer were made in concentrations of 0, 19, 25, 38, 50, 75, 100 and 150 ng/ml. 20 μl of each of these dilutions were added to 30 μl tris-buffer and 60 μl of the above mentioned enzyme preparation. The mixtures were incubated at 37°C for 2 hours. The subsequent procedure was performed as described by Poulsen (5).

RESULTS

1 To samples of 5 ml pooled human plasma were added human standard renin (research standard National Institute for Medicine Research, Mill Hill, London) in concentrations indicated in Fig. 1 The samples were incubated for three hours at 37 C a.m. (1) The angiotensin formed was determined as described above. The good correlation between added standard renin and angiotensin II formed is seen in Fig. 1

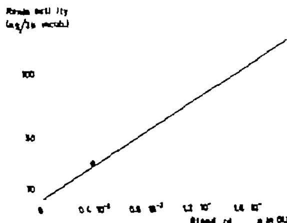


Fig. 1. The correlation between standard ren... to pooled human plasma (abscissa) ... angiote... after 3 hours incubation (ord...

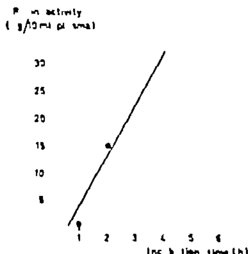


Fig. The correlation between incubation time (abscissa) and angiotensin formed (ordinate).

II. To samples of 5 ml pooled human plasma were added in each case 0.4×10^{-3} Goldblatt units of standard renin. The samples were incubated for 1, 2, 3 and 4 hours respectively. Fig. _ shows the linear relationship between incubation time and amount of angiotensin II formed.

DISCUSSION

Hollemans et al. ( ) demonstrated that the incubation product of the Boucher method is angiotensin I. Having at our disposal a well functioning radio-immunoassay for angiotensin II, it was decided to convert angiotensin I to angiotensin II. This also makes it possible to measure circulating angiotensin concentration as well as renin activity by the same radio-immunoassay. The conversion of angiotensin I to II was performed by a preparation of human plasma containing converting enzyme, in which angiotensinase activity was sufficiently diminished. A 100% conversion of angiotensin I is not obtained, but a correction is evaded by the formation of a standard curve with synthetic angiotensin I—identical to human angiotensin I—converted to angiotensin II in the same way.

Hollemans et al. (3) have also coupled immunoassay *to the method of Boucher. This group* applied antibodies to angiotensin I. After the addition of a step of acid precipitation of the plasma samples and alcohol precipitation of the eluate from the resin columns, Hollemans et al. (3) succeeded in removing substances interfering with radio-immunoassay by diminishing the affinity between angiotensin I and antibody. Similar problems have not been encountered in the present procedure.

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MORTALITY OF HEART BLOCK COMPLICATING ACUTE MYOCARDIAL INFARCTION MANAGED WITHOUT ARTIFICIAL PACEMAKERS

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Abstract. Complete heart block has been observed in 42 (7%) of 603 patients with acute myocardial infarction (AMI) between 1966 and 1969 in this hospital. In addition 11 patients got their complete heart block after resuscitation and are excluded from this material. Of the 42 patients 24 were given isoprenaline and 10 insulin, glucose and potassium. No patients were treated with artificial pacemaker. Twenty-two (52.4%) of the patients with complete heart block died, compared to a total mortality of 30%. The most adverse prognostic factors were BP below 80 mmHg (12 patients) and syncope (12 patients). Of the 22 patients with posterior infarction 11 (50%) died, compared to 4 (33.3%) of the 12 patients with anterior infarction. Of the 8 patients with posterior and anterior infarction or uncertain localization of the infarct 7 (87.5%) died. Of the 22 patients who died 13 died of shock or cardiac failure, 4 of ventricular fibrillation, 4 of asystole and 1 of stroke. More information must be collected to demonstrate unequivocally the value of artificial pacemaker in the treatment of heart block in AMI.

It has been claimed that endocardial pacing reduces the mortality of heart block complicating acute myocardial infarction (AMI) from about 67–100% (8, 9) to about 28.8–49% (4, 7, 10, 12, 14). The medical management not including pacemakers has, however, also changed in the last decade, and the previous reports on mortality rates without pacemaker treatment may no longer be valid. Pacemaker treatment is not without complications and carries a certain mortality when used under these circumstances. Before adapting pacemaker treatment, we therefore found it necessary to reexamine the results obtained without artificial pacemakers.

MATERIAL AND METHODS

In a 4-year period between 1966 and 1969 603 patients with AMI were discharged from Rogaland Hospital, Medical Department. The diagnosis was considered to be established if pathological Q waves appeared on the ECG. Changes in the S-T segments and T waves suggestive of infarction or BBB were also accepted when accompanied by a significant and transient increase in the SGOT (15). The ECG site of infarction was classified as posterior if there were Q waves and S-T segment changes in leads III and aVF and anterior if these changes occurred in the chest leads with or without changes in leads I and aVL. ECG changes typical of AMI in other locations were classified as combined posterior and anterior infarctions or as infarction of uncertain location. Criteria for the diagnosis of first degree A-V block was prolongation of the PQ interval to at least 0.24 sec irrespective of heart rate. Second degree A-V block was defined as incomplete A-V block with dropped ventricular beats in the presence of sinus rhythm. Second degree A-V block was divided into two types: type 1 was defined as the Wenckebach phenomenon, while the definition of type 2 rested on the presence of a constant PQ interval for conducted sinus beats irrespective of the ratio of atrial to ventricular depolarization. The diagnosis of complete heart block was based on the presence of identifiable atrial depolarizations and total A-V dissociation.

The age and sex distribution and the mortality of the 42 patients with complete heart block are given in Table I. In 11 patients the A-V dissociation appeared for the first time during resuscitation from cardiac arrest. These patients are not included in this material.

During 1968 and 1969 most patients with AMI were kept under continuous cardiographic monitoring in the Coronary Care Unit for 4 to 7 days.

The medical treatment of the 42 patients with complete heart block was isoprenaline infusion in 24 cases, glucose-potassium-insulin infusion in 19 cases. In 18 cases no special drug treatment was judged to be necessary. Atropine was not used in our material and pacemaker treatment was not tried in any of the cases.

For the statistical analyses of the results the χ^2-test has been used.

RESULTS

Incidence of complete heart block

Complete heart block occurred in 39 (6.5%) of 600 patients with AMI. In addition 3 patients

Table I *Age and sex distribution and deaths among patients with complete heart block*

Age (y)	Male ( )		Female ( )		All pats. ( )	
	Total	Deaths	Total	Deaths	Total	Deaths
< 59	7	1	2	0	9	1
60	18	9	15	12	33	21
Total	25	10	17	12	42	22

were transferred from another hospital for treatment of complete heart block complicating AMI

Partial block prior to complete heart block

In 7 patients there were first degree and second degree type I block without progression to complete heart block. Of the 42 patients with complete heart block, 11 had first degree, second degree types 1 or 2 as the initial conduction defect.

Time of onset and duration of heart block

In 17 patients complete heart block appeared within the first .4 hours after the onset of the severe chest pain. The heart block was transient in all but 2 of the patients surviving. One of these patients had narrow QRS complexes and a total A V dissociation, and the rhythm has been unchanged for .5 years. The other patient had anterior infarction and was discharged with complete heart block and wide QRS complexes. He lied 5 5 months later Fifteen of the 2. patients who d ed had complete heart block at death.

Mortab nd location of infarct

Of the 1 patients with anterior infarction 8 had QRS duration of 0 1 sec or greater (Table II).

Year of occ rren and mortality rate

The mortality for all patients with AMI before 1968 was about 35 whereas it was 25% for the

Table II *Relationship between site of infarction and mortality rate in 4 patients ith complete heart block*

Site	N of pat	Deaths	Mortality
Inferior	22	11	50.0
Anterior	12	4	33.3
Anterior and inferior	5	5	100
Undetermined	3		66.7
Total	42	22	52.4

Table III *Year of occurrence and mortality rate for 603 patients with AMI and 42 patients with complete heart block*

Year	AMI			Complete heart block		
	No. of pats.	Deaths	Mortality	No. of pats.	Deaths	Mortality
1966	147	53	36.1	4	3	75
1967	145	50	34.5	15	11	73.3
1968	144	36	25.0	10	2	20.0
1969	167	42	25.1	13	6	44.2
Total	603	181	30.0	42	22	52.4

years 1968 and 1969 At the same time the mortality for the patients with complete heart block fell from about 74 to 35% (Table III).

Prognostic factors

Table IV indicates some possible prognostic factors grouped according to apparent order of importance.

Causes of death

There were 22 deaths among 4. patients (mortality 52.4%) Thirteen patients died of cardiogenic shock and heart failure. The remaining deaths were due to ventricular fibrillation in 4 cases, ventricular asystole in 4 and cerebral vascular injury in 1 case

Table IV *Prognostic factors in 42 patients*

Factor		No. of pats.	Deaths (n)	Deaths ( )	p
BP < 80 mmHg (known in 39 patients)	Yes	12	11	91.7	0.001 p 0.005
	No	27	9	33.3	
Syncope	Yes	12	9	73.0	0.05 p 0.1
	No	30	13	43.3	
Previous infarction	Yes	6	5	83.3	0.1 < p 0.2
	No	36	17	47.2	
Block within 24 h	Yes	17	10	58.8	0.4 p < 0.5
	No	25	12	48.0	
QRS 0.12 sec	Yes	19	11	57.9	0.5 < p 0.6
	N	23	11	47.8	
Pulse rate < 40	Yes	15	8	53.3	0.7 p 0.8
	N	27	14	51.9	

DISCUSSION

Grendahl and Sivertssen (4) found a mortality rate of 49% in 53 cases of complete heart block complicating AMI following pacemaker insertion. Similar results are reported by Sutton et al. (12) who had a mortality of 47.8% in 46 cases, Lassers and Julian (7) 47% in 51 cases, Scott et al. (10) 37% in 27 cases and Watson and Goldberg (14) 48.8% in 45 cases. In a collected series of 131 patients treated by internal pacing 65 died, a mortality rate of 50% (3). In a collected series of 270 patients treated without pacemaker 93 (54%) died (2), and in a similar series the mortality was found to be 58% (3). In our series 22 (52.4%) of the 42 patients with complete heart block died.

In our series anterior myocardial infarction had the lowest mortality. This is in contrast to most previous reports (3, 4, 7, 10, 12), except for the series of Strøde Nielsen et al. (11) in which only 2 of 7 patients with anterior infarction and complete heart block died. On the other hand the mortality in the cases associated with inferior infarction is high compared to series with pacemaker-treated patients. It is possible that better results would have been obtained if pacemakers had been used in this group. This view is in accordance with that of Watson and Goldberg (14).

The patient with permanent heart block and narrow QRS complexes probably has a destruction of the A V node. These cases are seldom seen (13). The other patient with permanent heart block and wide QRS complexes lived only 5.5 months. A similar result is reported by Lassers (5), only 3 patients with anterior infarction and complete RBBB surviving to be discharged. These patients died during the first year. There were no significant differences between the mortality of the patients with narrow and wide QRS complexes. These data appear to be in contrast to previous reports (3, 4, 7, 12, 13). A wide QRS complex does not necessarily mean an extensive infarction of the myocardium.

Death seemed to be more related to the overall severity of the infarct than to the heart block itself. In accordance therewith the causes of death were heart failure and shock in most of the patients dying with heart block.

BP below 80 mmHg and syncope were the most serious prognostic signs. Patients with normal mental function and good skin circulation usually have adequate cardiac output and normal BP and consequently a fair prognosis even if their AMI is complicated by heart block (6).

A diseased myocardium may be incapable of increasing its stroke volume, so that changes in cardiac output are completely dependent on heart rate (2, 6). Cardiac output can be increased by increasing the heart rate by artifical pacing or by drugs. The rate associated with the maximal cardiac output is found to be up to 120 min (6). Although isoprenaline has been found to be beneficial in patients with very slow ventricular rates and syncopes, the dosage is difficult to control without producing ventricular tachyarrhythmia. The effect of isoprenaline is unpredictable and may vary from one patient to another and in the same patient from time to time, suggesting difference or change in myocardial responsiveness to the drug. In some patients the effect of this drug on the idioventricular rate is insufficient to suppress the preexisting ectopic beats. We have seen these ectopic beats suppressed when isoprenaline was used in combination with lidocaine. One should distinguish between the ectopic beats caused by isoprenaline, preexisting irritability of the myocardium or slow basic rhythm with compensating ectopic pacemaker foci. These different types can be studied by careful examination of ECG recordings reproduced from a tape recorder. A better understanding of the arrhythmias associated with myocardial infarction and complete heart block has to be achieved before definite recommendations of the therapy can be given. It is possible that the treatment of this type of complete heart block has to be more differentiated. We believe that our medical treatment not including pacemakers has improved. Support for this view is given in Table III, but the number is too small for a reliable conclusion. In 1966 we had no intensive care unit, and the monitoring of the patients had just started. The number of patients with complete heart block in this year is small compared with the other years, probably indicating that only some of the blocks occurring were registered. On the other hand, increasing experience and knowledge of pacing will reduce mortality and Bouvrain (1) claims to have reduced the mortality from 60 to 32%.

Failure to classify cases in terms of factors besides heart block, which might influence prognosis, precludes any conclusion as to the value of medical treatment or pacemakers in heart block

with myocardial infarction. However we think that continued application of medical treatment only is justified in most cases. The problem is to identify the group of patients who will definitely benefit from pacemaker treatment.

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PROTECTION OF PHAGOCYTIZED BACTERIA AGAINST ANTIBIOTICS

A New Method for the Evaluation of Neutrophil Granulocyte Functions

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Abstract. Antibiotic-sensitive *Staphylococcus aureus* phagocytized by human neutrophil granulocytes were exposed to mixture of penicillin G and streptomycin in concentrations from 25 U (25 μg/ml) to 1000 U (1 000 μg/ml). Phagocytized bacteria were effectively protected from the antibacterial effect of the antibiotics. Considerable numbers of intracellular bacteria remained viable after 20 h exposure to concentrations of antibiotics that killed more than 99% of extracellular bacteria in less than 30 min. Killing of phagocytized bacteria by granulocyte enzymes as effectively inhibited by phenylbutazone. A new method is described for the evaluation of the number of viable intracellular bacteria, using phenylbutazone for the inhibition of intracellular killing of bacteria and high concentrations of antibiotics for the control of extracellular organisms. This method facilitates an in vitro evaluation of the phagocytic and bactericidal activities of neutrophil granulocytes.

In recent years several disease syndromes characterized by persistent or recurrent bacterial infections have been related to defects in either phagocytosis or intracellular killing of bacteria by neutrophil granulocytes (6, 7 10 15 16, 17 18). Diagnosis of these disease syndromes requires analyses of both phagocytic and bactericidal activities of the granulocytes. Many of the analyses of these activities are made in vitro, in culture media prepared with antibiotics to prevent multiplication of extracellular organisms (2, 6, 8, 11 14). The inactivation of extracellular bacteria with antibiotics facilitates long-term studies of intracellular organisms and would reveal a defect in bactericidal activity which ordinarily would be obscured by extracellular multiplication. However little is known about the effect of antibiotics on intracellular bacteria, and differentiation between bacterial inactivation by antibiotics versus granulocytes may be difficult.

Our study presents evidence that phagocytized *Staphylococcus aureus* is protected for as long as 20 hours from concentrations of antibiotics which would kill more than 99% of extracellular bacteria in less than 30 min. Based on these findings a new method is developed for the determination of the number of viable intracellular bacteria, facilitating a precise in vitro evaluation of the phagocytic and bactericidal activities of neutrophil granulocytes.

MATERIAL AND METHODS

Only disposable plastic materials and siliconized glassware were used.

Leukocytes

Blood samples were obtained from patients and normal individuals. The preparation of leukocytes was performed by the cell separation technique described by Bøyum (4) with minor modifications. Heparinized venous blood (10 U heparin/ml blood) was collected by syringe and layered on top of two-phase cell separation system in Falcon disposable plastic tubes (16 150 mm). The cell separation mixture contained 10 parts Isopaque (Natrium N-methyl-3,5-diacetamido-2,4,6-triiodobenzoate) 33.9% (obtained by dilution with distilled water of Isopaque® 75% Nyegaard, Oslo, Norway) and 20 parts dextran 6% (obtained by dilution with distilled water of Dextran 500, Pharmacia, Uppsala, Sweden). Volumes giving blood columns of 60 to 70 mm were employed, and the volume of Isopaque-dextran mixture as 3/4 of the blood volume. Ten minutes after the erythrocytes had passed the interface between plasma and Isopaque-dextran (usually after 40–50 min), the leukocyte-rich plasma layer was pipetted off and centrifuged at 500 g for 5 min. The cellular pellet was twice washed in 5 ml heparinized saline (1 U heparin/ml saline) by centrifugation at 500 g for 5 min. After the final centrifugation differential count was performed and the cells were resuspended in Hank's balanced salt solution containing 0.1% gelatin to make concentrations

with myocardial infarction. However we think that continued application of medical treatment only is justified in most cases. The problem is to identify the group of patients who will definitely benefit from pacemaker treatment.

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bacteria suspension (10 bacteria/ml). The tubes were incubated at 37 C. Samples (1/100 ml) were removed at prescribed intervals and twice washed in Hank's balanced salt solution. The deposit was suspended in 1 ml distilled water to facilitate osmotic disruption of the leukocytes. Quantitation of viable bacteria in this final suspension was done by a standard pour plate technique using Penn-assay agar

In the tests containing bacteria suspension, rapid killing of bacteria was observed, and after incubation for 60 min more than 99% of the bacteria had been killed in the tests containing more than 100 U penicillin G and 100 μg streptomycin (Fig. 1). In contrast, the reduction in viable organisms in the granulocyte-bacteria tests was very slow and considerable numbers of bacteria remained viable after incubation for 20 h. The more pronounced reduction in viable bacteria during the early phase of incubation of the granulocyte-bacteria tests is due partly to inactivation by the antibiotics of contaminating extracellular bacteria (5 14 21). Only minor differences in reduction of viable bacteria were observed between tests containing large and small amounts of antibiotics, indicating that the decrease in viable bacteria was the result of the killing by granulocytes rather than the penetration of antibiotics into the leukocytes in quantities sufficient to cause bacterial destruction.

Experiment 2

To support the evidence that the decrease in viable bacteria in the granulocyte-bacteria tests was a result of leukocyte killing rather than an effect of the antibiotics, 2 mg phenylbutazone (Geigy Basel, Switzerland) was added to tubes containing 1 ml granulocyte-bacteria suspension in order to inhibit intracellular killing of bacteria by leukocyte enzymes (20). The leukocytes in the granulocyte-bacteria suspension used had been exposed to the bacteria for only 6 min and possessed marked bactericidal activity (19). Control tubes and tubes containing phenylbutazone and amounts of penicillin G and streptomycin as indicated in Fig. 2 were incubated at 37 C. Samples (1/100 ml) were removed at prescribed intervals and quantitation of viable bacteria was performed as described in experiment 1

A significant reduction in viable bacteria was

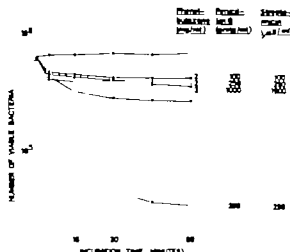


Fig. 2 The effect of antibiotics and phenylbutazone on phagocytized and extracellular contaminating *Staphylococcus aureus* (mean of three experiments).

observed in the test without phenylbutazone and antibiotics and no reduction in the test with phenylbutazone, demonstrating the effective inhibition by phenylbutazone of intracellular killing of bacteria (Fig. 2) In the tests with phenylbutazone and antibiotics minor reductions in viable bacteria were observed during the early phase of incubation due to inactivation by the antibiotics of contaminating extracellular bacteria. Later the number of viable bacteria in these tests remained fairly constant, and only minor differences could be demonstrated between tests with markedly different concentrations of antibiotics, indicating that antibiotics did not significantly influence the survival of intracellular bacteria even in concentrations that would have killed more than 99% of extracellular bacteria in less than 30 min. A marked reduction in viable bacteria was observed in the test containing antibiotics but no phenylbutazone demonstrating the added effects of intracellular killing of bacteria by granulocytes and the extracellular killing by antibiotics.

Experiment 3

To substantiate the evidence that phagocytized bacteria are protected from the killing action of antibiotics, granulocytes were obtained from patients with fatal granulomatous disease of childhood (characterized by chronic suppurative lym-

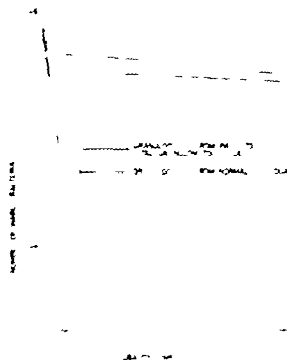


Fig. 3. Survival of phagocytized *Staph* ... exposed to 250 U penicillin G and 250 µg streptomycin

phadenitis, hepatosplenomegaly, skin sepsis, multiple organ abscesses, and the patients die at an early age)—a disease based on a defect of intracellular killing of bacteria (18). The leukocytes in the granulocyte-bacteria suspension used had been exposed to the bacteria for 6 min. Into separate tubes, each containing 1 ml granulocyte-bacteria suspension, were added 250 U penicillin G and 250 µg streptomycin. Two tests were performed with granulocytes from patients with fatal granulomatous disease and for comparison, two tests with granulocytes from normal individuals.

In contrast to the marked reduction in viable bacteria in the tests with granulocytes from normal individuals, only minor reductions could be demonstrated in the tests using cells from granulomatous patients (Fig. 3).

DISCUSSION

A number of extensive studies have been devoted to the interactions of bacteria and polymorphonuclear leukocytes (3). However, difficulties of methodology have resulted in divergent interpretations of experimental results. A major problem has remained the elimination of extracellular bacteria from the phagocytic system after a suitable period of incubation in order to measure the number of viable intracellular organisms. Previous workers have usually resorted to differential centrifugation in order to separate extracellular and intracellular bacteria. This procedure is not entirely quantitative and during the early phase of incubation, when the ratio of extracellular to intracellular bacteria is high, extracellular contamination may exceed 10% of the original inoculum and markedly obscure the determination of the relatively small numbers of viable intracellular bacteria (5, 14, 1). In addition bacteria adhering to the external granulocyte wall may not be eliminated by differential centrifugation. Finally, if the leukocyte suspension contains erythrocytes, which is usual when blood samples are used for the preparation of the granulocytes, immune adherence of bacteria to the erythrocytes may become a major problem.

To solve these problems several investigators have used antibiotics to inactivate extracellular bacteria, assuming that intracellular organisms would not be influenced by these drugs. This assumption has been supported by some investigators (1, 8, 14) but not all (9, 13). However the results of our studies indicate that even high concentrations of bactericidal antibiotics, which would kill extracellular bacteria in less than half an hour, did not influence phagocytized bacteria significantly. Therefore, in our opinion, control of extracellular bacteria by antibiotics should be possible without reducing the number of viable intracellular organisms.

During the early phase of incubation of granulocytes and opsonized staphylococci, rapid killing of intracellular bacteria occurs (19). This was clearly demonstrated in our experiment 2, where the granulocytes in the test suspensions had phagocytized for only 6 min and still possessed marked bactericidal capacity. A significant reduction in viable bacteria was observed in the control test (Fig. 2, second curve from the bottom) during the early phase of incubation. However these results were obscured by contamination with extracellular bacteria, and the relative reduction in viable intracellular bacteria was even more pronounced than visualized from the results of the control test. This was clearly demonstrated when streptomycin and penicillin were added to

the test suspension to eliminate extracellular bacteria (Fig. 2, bottom curve)

Inactivation of extracellular bacteria by streptomycin and penicillin, in concentrations not significantly influencing intracellular bacteria, takes about 15 min at 37 C. During this period the killing of intracellular bacteria by the granulocytes may markedly obscure the results especially during the early phase of interaction of granulocytes and bacteria. Therefore a method to determine the number of viable intracellular bacteria after a prescribed period of incubation requires not only control of extracellular bacteria, but also inhibition of the bactericidal activity of the granulocytes. As demonstrated in Fig. 4, phenylbutazone in a concentration of 2 mg/ml effectively prevented intracellular killing of bacteria. The combined use of phenylbutazone and antibiotics seems at present a valuable method for the determination of the number of viable intracellular bacteria and should be superior to methods using differential centrifugation.

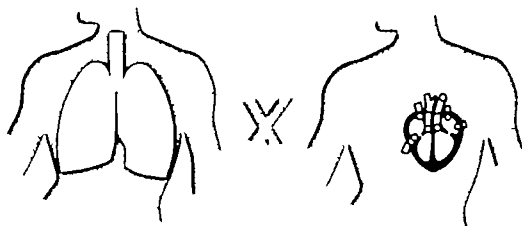
The phagocytic and bactericidal activities of neutrophil granulocytes can be expressed by the number of viable intracellular bacteria and the number of bacteria killed in an in vitro phagocytic system. The latter number can easily be determined by the original method of Maaløe (12) modified by Quie et al. (18). The number of viable intracellular bacteria is inversely proportional to the bactericidal activity of the granulocytes provided that phagocytic activity is normal. This was clearly demonstrated in our experiment 3 using granulocytes with a defect of intracellular killing but with normal phagocytic activity. Finally the number of bacteria phagocytized equals the number of viable intracellular bacteria and the number of bacteria killed in an in vitro phagocytic system.

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SULFONAMIDE HEPATITIS

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Abstract. Two cases of hepatitis induced by sulfonamides are described. The causative relationship was verified by the administration of a test dose. The administration caused in both cases a prompt appearance of clinical reaction followed by a rise of serum bilirubin and enzyme activities, which subsided within 10 days.

The early sulfonamides, particularly sulfanilamide, were reported to have hepatotoxic properties. In addition some of the earliest sulfonamide mixtures contained as vehicle diethylene glycol, which was confirmed to be a strong hepato- and nephrotoxic poison (7). Although the causal relationship of sulfonamides to hepatitis then reported may be questioned in many instances, the large accumulation of cases (96 before 1947) (4) strongly indicates the hepatotoxicity of older sulfonamides, although this was confirmed by a test dose in one case only (13).

The newer sulfonamides seem to be less hepatotoxic if the vague proof, the number of reported cases, is considered to reflect their hepatotoxicity (1, 2, 5, 8, 10, 15, 16, 17). In three cases the causal agent has been verified by the readministration of a test dose (4, 6, 9).

This report deals with two cases of hepatitis caused by sulfonamide-containing sulfamethoxypyridazine and sulfamethizole. The causal relationship was confirmed by a test dose in both cases.

CASE REPORTS

Case 1

A 68-year-old housewife was admitted to the hospital for jaundice, severe itching, dry mouth and progressive malaise. She had observed the jaundice, dark-red urine and pale stools for a few days before the admission.

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At the age of 13 she had been icteric, which had been regarded as infectious hepatitis. She had had no symptoms of liver or gall bladder disease until the present episode.

During the last ten years she had had several urinary tract infections which had been treated with unknown sulfonamide preparations without untoward effects. A year previously a sulfonamide containing sulfamethoxy*pyridazine and sulfamethizole (Sulf pral F)* had been administered for four weeks, and the same treatment had been repeated three months before the admission for another four weeks without untoward effects. Because the frequency and pain of micturition reappeared, the same sulfonamide was again prescribed and the patient had been taking two tablets twice daily for four weeks before the admission. There was no evidence of exposure to jaundiced persons, no injections were given, and alcohol ingestion was denied. Ten years earlier the patient had had several grand-mal seizures and since then she had taken diphenylhydantoin, 200 mg daily, without any symptoms of epilepsy. Otherwise the family and past histories were not contributory to the present illness.

On admission the patient was icteric and somewhat dehydrated. The BP was 150/90, pulse rate 78/min and rectal temperature 36.2°C. The liver was slightly tender on percussion, but the margin was not palpable. No arachnes, palmar erythema or spiders were observable. Blood Hb was 14.. g/100 ml, total WBC 6 400/mm³ with a normal differential count. The platelet count was 116 000/mm³. Eosinophil count was low, 22/mm³. The urine tests were positive for bilirubin and urobilinogen. Serum creatinine was 0.7 mg/100 ml. Urine cultures revealed a few coliform bacteria.

On admission serum bilirubin was 12.6 mg/100 ml (totally direct reacting). SGOT activity was 490 IU/l (upper normal limit 18), SGPT 700 IU/l (upper normal 16), and serum alkaline phosphatase (AP) 177 IU/l (upper normal 46). The subsequent course of these activities is shown in Fig. 1. Electrophoretic separation of AP showed the isoenzyme band at α-2-globulin, as in normal serum. Alkaline phosphatase was inactivated at 56°C as normal serum. Serum ornithine carbamoyl transferase was 1.48 IU/l (upper normal 0.40), serum amylase 148 (upper normal 170), and urine amylase 1 183 (upper normal 1 500). Total serum LDH activity was 420 IU/l (upper normal 180), with preponderance of LDH₅ isoenzyme in electrophoresis. Serum protein electrophoresis was within normal limits; total protein 6.2 g/l. Prothrombin time

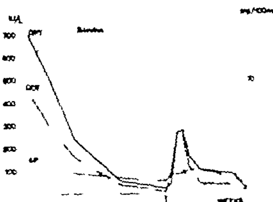


Fig. 1. Case 1. Serum enzyme activities (scale on the left) and serum total bilirubin (scale on the right) during treatment in the hospital. The arrow shows the time when a test dose was given.

as 76%. Australian antigen, measured twice, was negative as α_1-fetoprotein.

Liver biopsy performed two weeks after admission, showed swelling of parenchymal cells, with infiltration of lymphocytes and occasional eosinophils. PAS staining showed granulation. Neither bile lakes nor thrombi were seen. Fine-needle biopsy performed one day after admission, revealed some enlargement of the bile canaliculi in naphthylamidase staining as in acute bile stasis (18).

All medication was discontinued on admission and the fluid balance was substituted according to the laboratory values. All symptoms and liver function tests were normalized within 3–4 weeks. After having been without medication for ~3 weeks the patient had a grand-mal seizure. Phenobarbital and diazepam were administered because diphenylhydantoin has also been reported to cause hepatitis (3).

After the serum enzyme activities and bilirubin had become normalized, a test dose of two tablets of the same sulfonamide compound was given. Two hours later the patient felt heavy chills, dry mouth, signs of conjunctivitis, and the axillary temperature rose promptly from 36.4 to 39.1°C. Serum enzyme activities and bilirubin were augmented on the next day (Fig. 1). The symptoms subsided within some hours, and the enzyme values gradually became normalized over a period of about ten days. To test the possible role of diphenylhydantoin on the liver function the patient was given her earlier dose of this preparation without any side-effects. She had been taking this medication for eight months thereafter without symptoms. In oral cholecystography performed before discharge one gallstone was seen in the gallbladder but it has caused no symptoms so far.

Case 2

A 38-year-old female textile-worker was admitted to the hospital for jaundice and fever which had lasted for some days. She had never earlier been icteric. One year earlier she had been given a sulfonamide containing sulfamethoxypyridazine and sulfamethizole (Uro-Sulfa®) for some weeks for an urinary tract infection. For the same reason she had been taking the same sulfonamide for about two months before the present admission. Her past social and family histories revealed no clear contributory factors to the present illness.

On admission she was clearly icteric and had vague epigastric pain. Her rectal temperature was normal, and there were no signs of cardiac insufficiency. The BP, blood Hb and WBC were normal. The differential WBC revealed 6% eosinophils. The urine tests were positive for bilirubin and urobilinogen.

Serum total bilirubin was 8.7 mg/100 ml (5.4 reacting directly), SGOT 790 IU/l, SGPT 470 IU/l and serum AP 64 IU/l. LDH was 325 IU/l, and LDH isoenzyme activity had increased to 20% of the total activity. Serum OCT was 0.90 IU/l. Serum and urine amylase were normal. Serum total protein was 7.2 g/l, with no abnormalities in electrophoresis. Prothrombin time was 60%. Australian antigen and serum α_1-fetoprotein were twice negative.

Liver biopsy performed one week after the test dose administration, showed scanty infiltrations of round cells; no eosinophils were seen. There was neither cholestasis nor granulation.

The sulfonamide medication was discontinued on admission. When serum enzyme activities and bilirubin had returned to normal (Fig. 2) a test dose was given with two tablets of the same sulfonamide compound. Five hours afterwards the patient had heavy chills and her temperature rose from 36.3 to 39.2°C. Next day she had slight icterus and enrichment of serum enzyme activities. These subsided within ten days.

DISCUSSION

Sulfonamide hepatitis is classified as a hypersensitivity reaction (14) usually associated with occurrence of fever, skin rash, conjunctivitis, arthralgia and eosinophilia. The clinical picture may however be atypical in many respects. For instance, in the present cases the classical symptoms were absent and there were normal eosinophil counts in all eight differential counts of leukocytes made at the culmination of the symptoms.

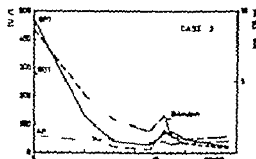


Fig. 2. The same parameters similarly presented as in case 1, Fig. 1.

The previous exposure to sulfonamides, the temporal association between administration of the drug and appearance of jaundice even with other manifestations of hypersensitivity are only suggestive of a causal relationship. The differentiation of a drug hepatotoxicity from a coincidental hepatitis is often impossible, although the drugs implicated in hepatic lesions can be classified according to the histologic picture (12) The exclusion of viral hepatitis by a negative Australian antigen, as in the present cases, is also vague evidence against a viral origin of the hepatitis.

If a sulfonamide is under consideration as the causal agent, there is the opportunity to verify this by a test dose Since the reaction is not dose dependent and has subsided soon after withdrawal of the drug, a challenge with a test dose seems to be justified. This must be weighed against the risk that the patient may be prescribed sulfonamides outside the hospital.

Because so few cases of hepatitis caused by the newer sulfonamides have been reported, it is a mere matter of speculation whether some sulfonamids are more hepatotoxic than others. Both long and short-acting sulfonamides have been reported to be hepatotoxic. Our patients were administered a combination of sulfamethoxypyridazine with a half-life of 35–40 hours and sulfamethizole with a half-life of about 2 hours. It would therefore be going too far to discuss whether the chemical structure the binding to serum proteins, the excretion rate or the acetylation ratio are responsible for their hepatotoxicity It is hoped that all cases of sulfonamide hepatitis will be registered so that their hepatotoxic properties can be evaluated.

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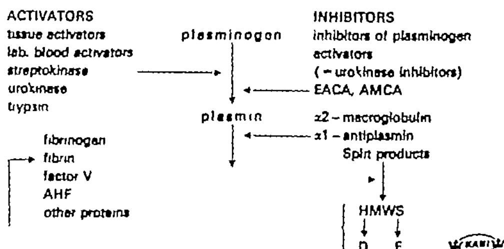
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Urinary tract haemorrhages may be caused by increased fibrinolytic activity Cyklokapron reduces or arrests fibrinolytic bleeding

In recent years fibrinolytic inhibitors have found wide spread use in a number of haemorrhagic conditions particularly in urinary tract haemorrhages and in connection with prostate surgery. Urine contains urokinase. This enzyme activates the conversion of the plasminogen present in the blood and blood clots into the proteolytic enzyme plasmin, which dissolves clots and thus sustains various types of haemorrhage in the urinary tract. Cyklokapron produces a haemostatic effect by counteracting the activity of urokinase.

The Swedish Investigators, Lennart Andersson and Inga Marie Nilsson, have obtained good clinical results by administering Cyklokapron to patients suffering from haemorrhages in the upper and lower urinary tract as well as postoperative bleeding following prostate surgery. Patients suffering from haematuria as a result of general fibrinolysis were also included in the investigation. Bleeding ceased completely in all the patients in the latter group, as was the case with most of the other patients.

the fibrinolytic system



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TRANSFERRIN EXCRETION IN PATIENTS WITH PROTEINURIA

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Abstract. Serum and urinary transferrin have been determined in 35 patients with renal disease and in 12 patients with urinary tract tumour. The relative content of transferrin in serum proteins did not differ significantly between patients with different diseases. The urinary protein content of transferrin was significantly higher in patients with glomerulonephritis than in patients with pyelonephritis. Patients with urinary tract tumour had lower transferrin content than patients with pyelonephritis, but this difference was not significant. The clearance of transferrin and the clearance of albumin were approximately equal in patients with renal disease and the correlation between the clearance values was highly significant. There was also significant correlation between the clearance of transferrin and that of IgG, but the deviation was greater. In patients with urinary tract tumour no significant correlation was found between the clearance of transferrin and that of albumin or IgG. Serum content and renal clearance of transferrin were not correlated to Hb concentration, serum iron, or creatinine, and no difference in this respect was found between patients with positive urine culture and non-infected patients. It is concluded that the renal clearance of transferrin depends on the molecular size. The nature of the renal disease causing proteinuria may influence the relationship between the renal excretion of transferrin and that of other proteins of different molecular size. Proteinuria originating from the lower urinary tract may have a different composition from the one of renal origin.

Protein clearance studies may be of diagnostic aid in glomerular disease (3, 6, 7, 8, 10). They are based upon the conception of the glomeruli as a molecular sieve (27), the filtration rate of the protein molecules being dependent upon the molecular size, and of a non-specific protein reabsorption in the tubuli (11). Transferrin has often been used as a reference protein in such investigations (6, 7, 12, 16, 24). However, the sieve theory is contradicted by the results of other studies. For instance, a great variation between the clearances of two molecules of equal magnitude, such as albumin and transferrin, has been reported in glomerulonephritis (1, 19), pyelonephritis (4, 5) and in normal subjects (21). This discrepancy in results could possibly be explained by methodological errors (19) and by complicated excretion mechanisms, including selective tubular reabsorption (2, 20).

The present study concerns the clearance of transferrin in patients with proteinuria. In order to investigate the influence of the molecular size on the protein excretion, transferrin clearance was compared with the clearance of albumin and with the clearance of a larger molecule, IgG. The results were analysed by conventional statistical methods. The transferrin excretion in glomerulonephritis, pyelonephritis and urinary tract tumour possibly representing three different pathogenic mechanisms of proteinuria, were compared. The influence of anemia, renal insufficiency and urinary tract infection on serum and urinary transferrin was also investigated.

MATERIAL

Out of 35 renal patients included in the study 13 had glomerulonephritis (histopathological diagnosis in 12), 12 pyelonephritis (histopathological diagnosis in 9), 6 renal amyloid (histopathological diagnosis in all) and 4 polycystic kidneys (histopathological diagnosis in 2, angiographic diagnosis in all). Twelve of these patients had normal serum creatinine and/or clearance values, and in 23 patients laboratory findings showed lowered glomerular filtration of varying degree. None had macroscopic hematuria. Ten patients (half the pyelonephritis cases) had positive urine culture at the time of the investigation.

Twelve patients had malignant tumours of the urinary bladder or renal pelvis. These were confirmed by biopsy in 11 and by cytology in all of the cases. The degree of malignancy varied (13). None of these patients had macroscopic hematuria, 10 had normal and 2 slightly elevated serum creatinine values. Urine culture yielded bacterial growth in 3 of these patients.

Table I. *Transferrin values in serum and urine, mean ± S.D.*

Group		Transferrin in serum mg/100 ml	% of total protein	Transferrin in urine mg/24 h	% of total protein
Healthy controls	49	289.6 ± 49.2	3.74[a]		
Glomerulonephritis	13	186.7 ± 74.5	3.55 ± 1.01	518.8 ± 464.3	5.51 ± 1.41
Renal amyloid	6	144.2 ± 48.8	2.60 ± 0.72	468.8 ± 316.3	6.26 ± 2.18
Pyelonephritis	12	211.9 ± 53.0	3.28 ± 0.96	87.0 ± 51.9	3.63 ± 1.05
Polycystic kidneys	4	168.8 ± 44.0	2.43 ± 0.66	34.8 ± 27.8	2.22 ± 1.00
Urinary tract tumour	1	260.0 ± 54.3	3.39 ± 0.73	78.8 ± 101.8	2.63 ± 1.22

[a] Values of transferrin and total protein were not determined in the same individuals.

All patients had proteinuria exceeding 0.4 g/l. Collection of samples and concentration of urine were performed as described earlier (14).

METHODS

Protein concentration in serum was determined by the biuret method and in urine, before and after concentration, by the Kjeldahl method. Albumin was determined after electrophoretic separation of the proteins in celluloseacetate gel (15) as described earlier (14). Transferrin and IgG were determined by the immunodiffusion method of Mancini et al. (17), using monospecific commercial antisera and purified proteins (18, 25) as standard antigens. The anti-IgG serum was specific to the γ-chains of the molecule. All sera and concentrated urines were investigated by immunoelectrophoresis, using antisera against human serum proteins and transferrin.

The relationship between the renal clearance of transferrin and that of albumin or IgG was studied by regression analysis. The clearance values of each protein showed skew distribution in the clinical material, some patients having very high values with a wide range and others having rather low values with little variation. By using the logarithms of the clearance values, normal distribution was obtained. Thus, in the equation used in the regression analysis

$$y = bx + a$$

y is the log of the renal clearance of transferrin and x is the log of the clearance of albumin or IgG.

RESULTS

Transferrin values in serum and urine in the different groups of patients are shown in Table I. Patients with glomerulonephritis or pyelonephritis, but not patients with urinary tract tumour, had significantly lower values of serum transferrin than healthy controls ($p < 0.001$). This difference was at least partly explained by a difference in

Table II. *Results of regression analysis, $y = bx + a$, where y means log clearance transferrin and x means log clearance albumin*

Patient group		Regression coefficient (b)	S.D.	Correlation coefficient (r)	t-value (b and)	a
All renal patients	35	1.059	0.026	0.990	40.09	0.091
Glomerulonephritis	13	1.042	0.047	0.989	22.08	0.064
Pyelonephritis	12	0.989	0.066	0.978	14.97	0.001
Urinary tract tumour	*12*	*0.610*	*0.320*	*0.516*	*1.91*	*0.306*

Table III. *Results of regression analysis, $y = bx + a$, where y means log clearance transferrin and x means log clearance IgG*

Patient group		Regression coefficient (b)	S.D.	Correlation coefficient (r)	t-value (b and)	
All renal patients	35	1.016	0.083	0.905	12.23	0.372
Glomerulonephritis	*13*	*0.834*	*0.111*	*0.915*	*7.51*	*0.879*
Pyelonephritis	12	0.822	0.153	0.862	5.39	0.635
Urinary tract tumour	12	0.635	0.307	0.560	2.14	0.404

total amount of serum proteins, since the amount of serum transferrin, expressed in per cent of total protein, did not differ significantly between patients with glomerulonephritis, pyelonephritis and urinary tract tumour. The other patient groups were too small to be included in this comparison.

The transferrin excretion in urine varied with the degree of proteinuria. The relative amount of transferrin, expressed in per cent of the urinary protein, differed between the different groups. Patients with pyelonephritis or urinary tract tumour had a significantly lower content of transferrin than patients with glomerulonephritis ($0.001 < p < 0.01$). In patients with urinary tract tumour the content of transferrin in the urinary protein was lower than in patients with pyelonephritis, but the difference was not significant.

Tables II and III show the values obtained for the regression coefficient (b), the correlation coefficient (r), and a in all renal patients and in the patient groups with the diagnoses of glomerulonephritis, pyelonephritis or urinary tract tumour. The values for each patient are also shown in Figs. 1 and 2.

When the clearance of transferrin and albumin are compared (Table II and Fig. 1) the regression coefficient is very close to 1.0 in the patients with

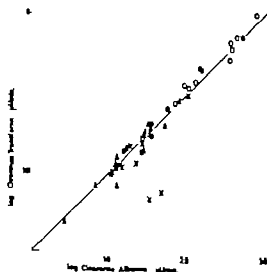


Fig. 1. The relationship between log clearance transferrin and log clearance albumin. The line represents the equation $y = x$, see text. Each symbol represents one patient: ○ glomerulonephritis; ● renal amyloid; △, pyelonephritis, ▲ polycystic kidneys; urinary tract tumour.

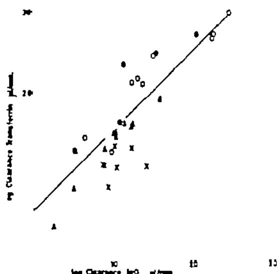


Fig. 2. The relationship between log clearance transferrin and log clearance IgG. The line represents the equation $y = +0.57$ see text. Each symbol (as in Fig. 1) represents one patient.

renal disease. In the well-defined groups of glomerulonephritis or pyelonephritis it does not differ significantly from 1.0. When all renal patients are included in one group, the difference is just outside the significant level. Assuming that the regression coefficient b equals 1.0, the difference between log clearance transferrin and log clearance albumin is constant, implying that the ratio between the two clearance values is constant, regardless of the degree of proteinuria. The value of a does not differ significantly from 0 in patients with renal disease, indicating that the ratio between the two clearance values is 1.0.

It will be seen from Table II that, when log clearance transferrin and log clearance albumin are compared, the correlation coefficient is very high. In Fig. 1 a line is drawn showing the regression line when b equals 1.0 and a equals 0. The values obtained in the renal patients are well adjusted to this line. As will be seen from Table II and Fig. 1 no corresponding relationship between the clearance of transferrin and that of albumin is found in the proteinuria of patients with urinary tract tumour.

A corresponding analysis of the relationship between the renal clearance of transferrin and the clearance of IgG is reported in Table III and Fig. 2. The regression coefficient does not differ

significantly from 1.0 In the patients with renal disease but the deviation is greater The correlation is significant. This means that the ratio between the clearance of transferrin and the clearance of IgG is more or less constant in these patients, regardless of the degree of proteinuria. The mean value of *a* for the patients with renal disease is 0.57 If the regression coefficient *b* equals 1.0 the difference between log clearance transferrin and log clearance IgG is 0.57 and the ratio between the two clearance values is approximately 3.7 The line drawn in Fig. 2 represents the regression line with the above mentioned values for *a* and *b*

In the comparison between the clearance of transferrin and the clearance of IgG no significant correlation was found in the group of patients with urinary tract tumour

No correlation was found between transferrin in serum, expressed in mg/100 ml and in per cent of total serum proteins, or transferrin clearance expressed in per cent of albumin clearance, and Hb concentration, serum iron or serum creatinine. This held true whether each group of patients was tested separately or all patients were analysed in one group. No significant difference in these transferrin values was found when patients with positive urine cultures were compared with other patients with the same diagnosis but without signs of urinary tract infection.

DISCUSSION

The content of transferrin in urinary protein was higher in patients with glomerulonephritis than in patients with pyelonephritis or urinary tract tumour In a previous study (14) patients with pyelonephritis were found to have a relatively large urinary excretion of β-globulin in comparison with patients with glomerulonephritis, and immunoelectrophoresis against anti-human plasma protein serum showed predominance of a transferrin line in the β-globulin region. This finding disagreed with results reported by others (2., .6), namely a transferrin line described as weak and atypical in pyelonephritis. Immunoelectrophoresis, however is not a quantitative method. In the present investigation, also, concentrated urine from patients with pyelonephritis showed a strong transferrin line when tested against anti-human plasma protein serum or monospecific anti-transferrin serum. The results indicate a qualitative difference between glomerular and tubular proteinuria, as has been shown in several previous investigations (23). Patients with urinary tract tumour seem to have a proteinuria that differs from the one found in glomerulonephritis and pyelonephritis patients, probably because of a high content of immunoglobulins (13).

The results of the statistical analysis show a similar clearance of transferrin and of albumin in patients with renal disease. This conclusion is based upon the assumption of a regression coefficient of 1.0 in the correlation between the logarithms of the clearance values. This could not be proved statistically for all the renal patients (Table II). However the value obtained is very close to 1.0 in patients with glomerulonephritis or pyelonephritis it did not differ significantly from 1.0 and the clearance results fit well to a line drawn on the assumption of a regression coefficient of 1.0 (Fig. 1). Considering the possibilities of experimental errors, the present results strongly indicate a similar rate of excretion for the two molecules in patients with renal disease.

Approximately the same clearance values for albumin and transferrin in renal patients have been reported in earlier investigations (3 10, 17). However other studies have shown very divergent excretion rates (1 4 5 19) For instance, in patients with proliferative glomerulonephritis the transferrin clearance/albumin clearance ratio varied between . and 800% (1). This great discrepancy in results is hard to understand, since the same or similar techniques have been used in most studies.

There was also a good correlation between the clearance of transferrin and the clearance of IgG in patients with renal disease, although the deviations were greater (Table III, Fig. 2). This is to be expected since the molecule of IgG is approximately twice as big as that of transferrin and the patients represent different types and stages of renal disease. The clearance rate of transferrin was on an average 3.7 times that of IgG, indicating a rather high selective index (7) or a nonselective type of proteinuria in the clinical material. Attempts were also made to correlate the clearance of transferrin to that of larger molecules, such as IgM or α-2-macroglobulin. However the variations in this heterogeneous group of patients were very pronounced when two molecules of

such different size were compared. Several patients showed no excretion at all of the macromolecules. A statistical analysis was not made.

Some of the patients with urinary tract tumour had an equal excretion of transferrin and albumin (Fig. 1), but as a group these patients had no significant correlation between the clearance of transferrin and the clearance of albumin or IgG. This indicates a different type of proteinuria in these patients, compared with patients with renal disease.

The correlation between Hb or serum iron concentration and serum transferrin content could have been influenced by several factors, such as blood transfusions given to some patients. No correlation was found. Nor was the degree of uremia, expressed as serum creatinine value, correlated to serum transferrin, which is in agreement with results from other investigations (9), or to the clearance of transferrin.

No difference in serum content or clearance of transferrin was found when patients with urinary tract infection were compared with non-infected patients. Ten out of 13 patients with positive urinary cultures had elevated serum creatinine values. Burdon (5) showed an increased transferrin clearance in patients with urinary tract infection compared with controls, but the difference was significant only in patients without renal insufficiency.

The results of the present investigation thus suggest that the renal excretion of protein molecules such as transferrin, albumin and IgG is dependent on the molecular size. Albumin and transferrin were excreted at the same rate. Larger molecules were excreted more slowly and the correlation in excretion rates may be influenced by the nature of the renal damage causing proteinuria. Such an influence appeared probable, since the proteinuria contained more transferrin in patients with glomerulonephritis than in patients with pyelonephritis. Proteinuria originating from the lower urinary tract seemed to have a different composition, the molecular size being of less importance. Extrarenal factors such as anemia, sideropenia and infection in the urinary tract had no influence on the excretion of transferrin.

ACKNOWLEDGEMENT

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EVALUATION OF THE FUNCTIONAL IMPORTANCE OF ATHEROSCLEROTIC OBLITERATIONS IN THE AORTO-ILIAC ARTERY BY PRESSURE/FLOW MEASUREMENTS

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Abstract. Arterial BP has been measured indirectly in the arm and intraarterially in the common femoral or external iliac artery of 23 patients with atherosclerosis obliterans and 2 patients with normal arteries. Blood flow in calf at rest and during reactive hyperemia has been measured by the use of venous occlusion plethysmography. The results of the pressure/flow measurements have been correlated to arteriographic findings. A systolic BP more than 10 mmHg lower in the common femoral or iliac artery than in the arm at rest and/or during reactive hyperemia was found to indicate atherosclerotic irregularities of functional importance. The results were found to be more informative if the ratio pressure drop/peak flow ("stenosis index") could be calculated. A stenosis index of more than 2 was found in patients with serious changes in the aorto-iliac arteries. The pressure measurements performed were found to give valuable information for the decision where to operate in cases of combined aorto-iliac and femoropopliteal obliterations. They may be made in connection with the arteriography as a supplement to the radiologic examination.

During the last years several authors have called attention to the significance of aorto-iliac stenoses in producing intermittent claudication (1, 4, 13). It has been emphasized that important aorto-iliac obstructions can exist, which is not apparent either at clinical examination or on an arteriogram (4, 13). Particularly in patients with atherosclerotic irregularities in the aorto-iliac arteries combined with marked stenosis or occlusion of the superficial femoral artery it is difficult to predict the functional significance of the proximal changes and to decide where to operate. In cases of significant proximal artery changes aorto-iliac reconstruction is recommended as the procedure of choice (1, 4, 9, 13). Admitting that a one-plane arteriogram may be inconclusive concerning the capacity of the iliac arteries, the possible need for objective supplemental tests should be examined.

In a steady streamlined flow the velocity of fluid increases as the cross-sectional area decreases in a stenosis. When the velocity increases, the pressure drops and a pressure gradient across the stenosis is produced (7). The gradient varies with the flow through the stenosis. In the present study measurements of the systolic arterial BP gradients across aorto-iliac artery obliterations have been used to evaluate the functional significance of arterial obliterations, particularly in patients with combined aorto-iliac and femoral artery disease. The results of the pressure measurements have been correlated to arteriographic findings.

MATERIAL

The study comprises 23 patients with atherosclerosis obliterans (mean age 58.7 years, range 37–74) and 2 patients (aged 51 and 70 years) without symptoms or signs of peripheral arterial disease. A thorough clinical examination and arteriography were performed in all patients with peripheral arterial disease to evaluate the accessibility for direct arterial surgery. In the 2 patients with arteries assumed to be normal, no arteriography was performed.

The state of the arteries judged from the arteriograms was classified, independently of the pressure/flow results, into the following types: type I, slight atherosclerotic changes which do not significantly reduce the arterial dimension; type II, moderate atherosclerotic changes with minor narrowing of the arterial diameter but without severe stenosis; type III, extensive and widespread changes with one or more stenoses of probable functional importance; type IV, subtotal stenosis or complete occlusion. The changes in the lower part of the aorta and the iliac arteries (in the sequel named proximal arteries) and the changes in the femoral and popliteal arteries (in the sequel named distal arteries) were classified similarly.

Patients with normal peripheral arteries. In 2 patients with normal arteries the systolic BP in the iliac artery was found to be higher than the systolic arm BP at rest. During reactive hyperemia no pressure drop was found.

Postoperative compared with preoperative results are presented in Table II. Three patients (nos. 3, 4 and 6) were successfully treated for their femoral artery occlusions by a femoropopliteal by-pass operation. In none of them did the restitution of satisfactory peripheral outflow result in a pressure drop across the proximal arteries. Despite a considerable increase of blood flow during reactive hyperemia after operation the pressure difference did not differ significantly from the preoperative results.

Endarterectomy of the iliac arteries was performed in 5 patients (nos. 11, 12, 14, 17 and 21). Four of them became free of symptoms and 1 (no. 11) improved. Postoperative pressure measurements, which were performed in 4 of them showed a pressure decrease of less than 10 mmHg during reactive hyperemia, despite the fact that 3 patients had a considerably higher flow. In 1 patient (no. 17) the postoperative peak flow value was only one half of the preoperative value. A considerably lower systemic BP may be the explanation (105/60 during the postoperative study compared with 165/90 during the preoperative study).

Comparison of arteriographic findings, pressure/flow measurements and pulse palpation

The pulsation in the groin was found to be reduced in all patients with arteriographic changes of types III–IV in the proximal arteries. In 5 of 8 patients with changes of type II the pulsation in the groin was found to be normal. None of them had a pressure drop exceeding 10 mmHg at rest. The atherosclerotic changes were found to be of functional significance in of them on the basis of the pressure drop which occurred during reactive hyperemia. In 2 of 6 patients without significant atherosclerotic changes in the proximal arteries judged from arteriography and pressure/flow measurements the pulse was judged to be slightly reduced.

COMMENTS

The study shows that measurements of the pressure gradient across the aorto-iliac arteries may be of value in predicting the functional importance of stenosis in these arteries.

The fact that the indirect systolic arm BP has been used in calculating the pressure gradient constitutes a source of error. Comparison of indirect and direct measurements of arterial BP shows somewhat different results. Most authors agree that the systolic pressure is commonly underestimated and diastolic pressure overestimated by the indirect method in adults of normal weight, while in the very obese both values will be overestimated (10, 12). The underestimation of the systolic pressure increases with increasing peripheral vascular resistance (3, 6). Intraarterial pressure measurements in supine subjects have shown that the systolic pressure in the femoral artery is usually about 20 mmHg higher than in the axillary artery (5, 12). Decreased flow velocity just proximal to arterial obliterations, differences in the rigidity of the artery wall and reflection of pressure waves from the main artery occlusions are factors which may have contributed to the discrepancies between the intraarterial and indirect systolic BP measurements in some of the patients in the present study. In none of the patients were arterial obliterations demonstrated in the arteries to the upper extremities.

The fact that the intraarterial systolic BP in the groin may be found to be considerably higher than the indirect arm BP implies that iliac artery stenosis of functional importance may exist despite no or even a negative pressure difference. In such patients the functional evaluation of the proximal arteries depends on the local pressure alterations obtained at different flow rates. The present study shows that significant proximal artery disease, which is not manifested during measurements at rest, may be demonstrated during reactive hyperemia. In patients in whom a pressure gradient across the proximal arteries becomes apparent only during reactive hyperemia, quantitative flow measurements are necessary to evaluate the pressure drop.

Ideally the pressure gradient should have been measured intraarterially by simultaneous measurements proximal and distal to the arterial obliteration, as done peroperatively by Wesolowski et al. (13). In the present study we intended to find a simple and reliable test for practical application which can be used as a routine examination before operation. Pressure measurements may be

performed in connection with the arteriography and should in our opinion be made routinely as a supplement to the radiologic examination.

The findings on pulse palpation are not easy to estimate without considerable experience. Very often the results are found not to be in agreement with the arteriographic findings (11). Palpation of normal pulsation at rest does not exclude proximal artery disease (2, 7, 13) as also demonstrated in some of the patients in the present study in whom diminished pulsation might have been obtained during reactive hyperemia (7).

CONCLUSIONS

A systolic BP gradient of more than 20 mmHg across the iliac arteries indicates severe arterial obliterations and a gradient of more than 10 mmHg indicates artery lesions of functional importance.

Patients with no obvious pressure gradient at rest may have arterial changes of functional importance. A pressure gradient may appear during higher flow as during reactive hyperemia. A pressure decrease during reactive hyperemia of 10–20 mmHg or more indicates artery lesions of functional importance. Even a pressure fall of only 5–10 mmHg during reactive hyperemia may indicate artery disease of functional importance if obtained in patients with low reactive hyperemia flow.

The "stenosis index" is usually higher than 2 in patients with arterial obliterations of functional importance. However, even an index of 1.5–2 may indicate artery disease of functional importance.

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MYOCARDIAL INFARCTECTOMY

A Clinical Study of Five Patients

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Abstract. The effect of surgical infarctectomy 1–9 weeks after acute myocardial infarction (AMI) has been evaluated in five patients with serious complications such as progressive heart failure and recurring tachyarrhythmias. Two patients had ventricular septal rupture as well. The diagnosis of left ventricular akinesis with ineffective motion of the myocardial wall was established by cine-ventriculography. All patients survived the operation. One did not regain consciousness and died from cerebral embolic disease after 1 week and another had sudden cardiac arrest and died 1 / weeks after the operation. The remaining three patients are all clinically well compensated 6 to 12 months after the operation and are at present living fairly normal life. Cardiac catheterization in one case showed much improved left ventricular function.

In 1947 Murray (11) proposed an emergency operation as a method to improve the chance of survival from the effects of massive myocardial infarction by resection of the infarcted area. His statement was based on satisfactory surgical results of experimentally induced infarctions in animals. More recently it has become evident that surgery with "infarctectomy" may be helpful in the treatment of complications of AMI in man. Candidates for surgical treatment are patients seriously ill from progressive cardiac failure, uncontrolled ventricular arrhythmias and ruptured ventricular septum with marked shunting. Successful resection of old infarction in patients with chronic postinfarction cardiac failure has been reported by several authors (10, 13). In these studies the surgical intervention took place several months to more than one year after the acute coronary artery occlusion. Emergency infarctectomy within the first weeks following a coronary occlusion was performed in four patients reported by Heimbecker et al. (7) in 1968. Three patients had repair of perforated ventricular septum in addition. Of the four patients one lived in well-being for more than one year.

The present report describes the clinical experiences with infarctectomy in five patients with AMI associated with cardiac failure or ventricular fibrillation resistant to medical therapy. Two cases had in addition ventricular septal rupture.

MATERIAL

In this series there were four men and one woman, aged 45–75 years. On admission the diagnosis of AMI was confirmed by ECG and serum enzyme changes.

In two patients the initial hospital course was complicated by ventricular septal rupture. This complication was confirmed by right-sided cardiac catheterization. The two patients were treated with fluid and salt restriction, digoxin and increasing doses of diuretics. Despite this therapy cardiogenic shock developed. In three patients ventricular arrhythmias requiring electrical cardioversion occurred several times in spite of lidocaine and other antiarrhythmic drugs. Hemodynamic deterioration gradually developed with low cardiac output, profound hypotension and signs of impaired cerebral and renal circulation. The rationale of subjecting these seriously ill patients to extensive cardiac surgery with infarctectomy as based on the identification of non-contractile area on the anterior or lateral left ventricular wall by cine-ventriculography

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Table I. *Clinical experiences with infarctectomy in 5 patients*

Case no.	Age (y)	Sex	Age of infarct (weeks)	Description of operation	Results
1	67	♀	1	Infarctectomy and septal rupture repair	Death after 1 week
2	70	♂	3	Infarctectomy and septal rupture repair	Improved
3	62	♂	7	Infarctectomy	Improved
4	66	♂	7	Infarctectomy	Death after 1½ weeks
5	45	♂	9	Infarctectomy	Improved

OPERATIVE TECHNIQUE

The operations were carried out one to nine weeks post infarction. The patients were premedicated with pethidine and promethazine, and anesthetics used were diazepam and fentanyl (modified neurolept analgesia) in addition to N_2O

Infarctectomy was performed under total cardiopulmonary by-pass with a bubble oxygenerator primed with lactated Ringer's solution and 5% dextrose in water (2 1) and anesthetic drugs.

The extent of the left ventricular infarction was well marked in all the cases, either as a non-contractile area with discolouration (early postinfarction), or as a thinned out area which became umbilicated when the blood was drained from the left ventricular cavity (scarred area). After excision the edges were approximated by continuous eversion suture and a continuous over-and-over suture. If, in addition, a ventricular septal defect was present, this was closed with a dacron patch (two patients)

Until the morning after operation all patients were artificially ventilated with a volume-preset, time-cycled respirator (Cape or Engstrom), being kept comfortable with phenoperidine

RESULTS

The clinical experiences with infarctectomy in five patients with AMI are listed in Table I. One patient, a 67 year-old woman with septal rupture (case 1), did not regain consciousness after the operation, and died from cerebral embolic disease after 1 week. The immediate postoperative course in the other patients was benign. On the 10th post operative day a 66-year-old man (case 4) suddenly developed cardiac arrest and attempts at resuscitation were unsuccessful.

The remaining three patients have been followed for at least 6 months after the operation. They have progressed well, the cardiac state is compensated, and there have been no episodes with ventricular tachyarrhythmias. At present they are able to live a fairly normal life. Cardiac catheterization pre and postoperatively was performed in case 2. The hemodynamic findings, summarized in Table II correlate well with the subjective improvement in this patient.

DISCUSSION

Two basic mechanisms are usually regarded as responsible for the hemodynamic deterioration following AMI. Decreased contractility with ineffective motion of the infarcted segment of myocardium leads to a reduced cardiac output and increases the end-diastolic pressure and volume, resulting in a reduced mechanical efficiency of the ventricle. A decrease in blood pressure and increase in ventricle diastolic pressure also lead to a reduced coronary artery flow compromising the oxygenation of the myocardium and thus further accentuating the failure of the left ventricle. The focal asynergic area also appears to be a focus of electrical irritability and its removal may result in a more stable rhythm or make ventricular defibrillation more successful. In addition, the adjacent healthy myocardium may be adversely affected by toxic effects such as metabolic acidosis

Table II. *Pre- and postoperative catheterization data from case 2 (combined septal rupture repair and infarctectomy)*

	Pre-operative 12.1 1970	Postoperative 5.3.1970	
	Rest	Rest	Exercise (120 kg/min in 3.30 min)
Blood gases (HbO %)			
Brachial artery	96.0	94.9	
Pulmonary artery	84.8	64.6	45.8
Right atrium	50.0	62.9	
Coron. sin. dist.	8.0	27 7	
Superior cava	36 8	67.3	
Pressures (mmHg)			
Right atrium	21	9	12
Right ventricle	64/23	29/8½	
Pulmonary artery	61/36–47	25/12–18	30½/17½–24
Pulmonary capillary venous	35	10	15½, 15½
Blood flow (Fick)			
CO/CI		6.1/3.7	

arising from an increased lactate production in the peri-infarction zones (8, 12). When coronary flow is insufficient to meet myocardial oxygen demands, the most significant metabolic shift is inadequate aerobic energy production and stimulation of anaerobic glycosis.

Several authors have reported hemodynamic improvement following experimental infarctectomy in animals. Heimbecker et al. (6) found that maximum left ventricular stroke improved markedly after resection of the infarcted area in calves. Jude et al. (9) reported an increase in left ventricular efficiency in dogs following infarctectomy. Glass et al. (3) found that the cardiac output as well as the systemic blood pressure improved significantly following resection of experimentally induced infarction in animals. In man the hemodynamic studies published by Harman et al. (5) and Lajos et al. (10) showed that, after resection of old infarction, the majority of the patients improved in end-diastolic volume and pressure and in cardiac output. Similar postoperative improvements from early infarctectomy were also obvious on clinical bases in the three surviving patients described in this paper. The favourable effect on the hemodynamics was also evident from the postoperative catheterization data presented in Table II, although the additional hemodynamic advantages which may have ensued from the simultaneous closure of a septal rupture are difficult to evaluate.

The postulate that resection of a localized infarction might be used as a therapeutic means in the prevention of recurring ventricular tachyarrhythmias is founded on experiments in animals. A number of investigators have reported that hearts which fibrillated following production of an infarction could be more easily defibrillated following infarctectomy (1, 3, 6, 9). DeBakey et al. (2) published a case of acute infarction with ventricular fibrillation occurring during operation for valve prosthesis. Attempts at defibrillation were unsuccessful until the infarcted muscle was excised. Several aspects from the present study also tend to support the assumption that infarctectomy may reduce the incidence of potentially lethal arrhythmias. The absence of ectopic electrical activity in the postoperative period contrasted sharply with the recurring episodes with ventricular fibrillation prior to surgical intervention.

Deleterious effects of infarctectomy may ensue when extensive infarcted area is removed. In animals resection of more than approximately 30% of the left ventricular myocardium was followed by a marked fall in ventricular efficiency resulting in a progressive deterioration of the myocardial function (14). Preoperative localization and quantitation of abnormal myocardial wall motion seem necessary. Coronary angiography, cineventriculography and cardiac catheterization would be important, although in many cases the preoperative examinations must be kept at a minimum because of the labile state of the patient.

One should also keep in mind the possibility of performing an aorta—coronary by-pass with a saphenous vein-graft to improve myocardial blood supply in an attempt to reduce the infarcted area before a possible resection.

Surgery with resection of non-contractile area of myocardial infarction is still an experimental procedure. Much further work is needed, including clinical trials with controls, before definite conclusions can be drawn as to the advantages of this form of therapy. At present, in certain patients with myocardial infarction, especially where medical management has failed to control cardiogenic shock, heart failure or recurrent ventricular arrhythmias, early infarctectomy should be considered. In these patients the risk of surgery is assumed to be lower than the risk involved in medical management alone (4). Later death from coronary heart disease or heart failure will occur because of progress of the underlying disease. After the excision, however, the remaining ventricular myocardium may be left in a more favourable situation, which gives the patient a chance to live a more active life.

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IRON–TETRACYCLINE INTERACTION
EFFECT OF TIME INTERVAL BETWEEN THE DRUGS

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Abstract. Iron is known to interfere with the absorption of *simultaneously taken tetracycline derivatives. The possibility* to avoid this interaction, by observing a suitable time interval between the drugs, has been studied on human volunteers using ferrous sulphate and tetracycline. Three consecutive experiments were performed with 25, 20 and 15 volunteers from a pool of 38 healthy medical students. It was found that if iron was given not less than three hours before or two hours after tetracycline no significant interference occurred.

It has been shown that iron impairs the resorption of simultaneously taken oral doses of tetracycline derivatives to such an extent that therapeutically inadequate plasma levels may result (5). This drug interaction is probably due to the formation of chelates in the gastro-intestinal tract (1). In some cases, however, it would be of value if iron therapy could be continued during tetracycline treatment. Accordingly we have tried to establish the minimum time interval between intake of these drugs at which no interaction of consequence takes place.

MATERIAL AND METHODS

The study was performed on healthy medical student volunteers aged 20–24, weighing 60–80 kg. Three separate experiments were carried out according to the following plan:

Twenty-five subjects took 500 mg tetracycline with a glass of water (200 ml) after overnight fasting. A control group of five persons received no other drugs, but in the remaining four groups five subjects ingested 600 mg ferrous sulphate either *simultaneously or half an hour*, one, two and three hours before tetracycline intake. Blood samples were drawn from the cubital vein one and a half and three hours after tetracycline ingestion. The serum antibiotic levels were determined fluorometrically according to a method described by Kohn (4), which was found to be more suited to the handling of large numbers of samples than the bioassay previously used. The results *obtained with these two methods were found to agree* well.

Twenty subjects were given 500 mg tetracycline as above. Five of them had no other drugs, while in the remaining three groups five subjects each ingested a dose of 600 mg ferrous sulphate one, two and three hours after the tetracycline. Blood samples were taken three and six hours after ingestion of the tetracycline and analysed as described.

Fifteen subjects were given 500 mg tetracycline twice daily during four days, at intervals of 12 hours. The drug was taken between meals and the subjects were instructed *to avoid milk and dairy products. Five of them ingested* 400 mg ferrous sulphate simultaneously with each dose of tetracycline, while another group of five took an identical dose of iron two hours after the tetracycline. The remaining five persons served as controls and received no additional drugs. Serum antibiotic levels were determined on the second and fourth day of the test, three and eight hours after ingestion of the morning dose of tetracycline.

Throughout this study gelatine capsules of tetracycline chloride corresponding to 250 mg of free base and sugar coated tablets of 200 mg ferrous sulphate containing 40 mg *elemental* iron were used. When tested according to the British Pharmacopoeia, Appendix XXI (2), in 0.6 % hydrochloric acid, these capsules and tablets disintegrated within 8 and 38 min respectively. In each test group of five subjects the mean serum antibiotic levels were calculated with standard errors (S.E.), and Student's *t*-test was applied in the evaluation of the results.

RESULTS

When iron was ingested half an hour, one hour or two hours before tetracycline, the reductions in the plasma antibiotic levels were significant, with $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively. But if iron was taken three hours before tetracycline, the antibiotic levels were of the same order as those attained when no iron was used.

Table I. *Mean serum levels (± S E.) 1.5 3 and 6 h after a single dose of 500 mg tetracycline A dose of 600 mg ferrous sulphate was taken 3 2 1 and 0.5 h before simultaneously with, and 1 2 and 3 h after tetracycline*
Number of subjects in each group = 5

Time interval between iron and tetracycline	Serum tetracycline (µg/ml)		
	1.5 h	3 h	6 h
Iron 3 h before	1.94 ± 0.27	3.02 ± 0.28	
Iron 2 h before	0.52 ± 0.20	1.50 ± 0 70	
Iron 1 h before	0.63 ± 0.39	0.93 ± 0 49	
Iron 0 5 h before	0.40 ± 0.16	0.69 ± 0.21	
Simultaneous intake	0.29 ± 0.11	0.34 ± 0.09	
Iron 1 h after		1 70 ± 0.46	1.30 ± 0.37
Iron 2 h after		3 16 ± 0.19	2.56 ± 0.24
Iron 3 h after			2.44 ± 0.33
Tetracycline alone	2.14 ± 0.43	2.94 ± 0.35	2.30 ± 0.27

Iron given one hour after tetracycline resulted in serum antibiotic levels about 40 % lower than with tetracycline alone, but when iron was ingested two and three hours later no reduction was found. These results are summarized in Table I. To make the time relationships in the iron–tetracycline interaction as lucid as possible, the serum concentrations three hours after tetracycline ingestion are shown in Fig. 1 as a function of the time interval between intake of the two drugs. In this figure the commonly accepted minimum therapeutic concentration (M.T C) of 0 6 µg/ml is indicated. It must, however be borne in mind that higher concentrations may be needed to inhib t growth of less sensitive bacteria.

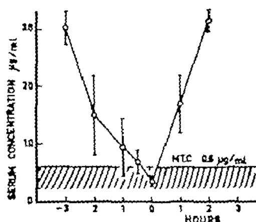


Fig. 1 Graphic presentation of the iron–tetracycline interaction. Ordinate: mean serum concentration 3 h after a single dose of 500 mg tetracycline. Abscissa: Interval between intake of tetracycline and 600 mg ferrous sulphate (left side, iron taken before: right side, iron taken after tetracycline).

In the subjects who were treated simultaneously with iron and tetracycline during four days, very low mean serum antibiotic levels were found. Both on the second and the fourth day the mean serum concentration eight hours after the morning dose was very near 0 6 µg/ml. The difference from the serum levels reached in the controls with tetracycline alone was highly significant ($p <$ 0.001). But if the iron was taken two hours after each tetracycline dose satisfactory serum levels were attained, which did not in practice differ from those reached in the controls, although a decrease could be noted (Table II).

DISCUSSION

According to this study the interaction between oral doses of iron and tetracycline can be avoided by spacing the intake of these drugs two to three hours apart. This time interval should be ample for ordinary therapeutic doses of iron, as it was found sufficient when the rather large dose of 600 mg ferrous sulphate was used.

It can also be seen that tetracycline absorption was impaired during a shorter time when iron was taken after the antibiotic than vice versa. This is probably due to the well known *incomplete* absorption of on (3), which leaves a large part free to interact. Tetracycline, on the other hand, is rapidly absorbed and thus escapes" a subsequent dose of iron.

When tetracycline and iron were taken simultaneously twice daily during four days, the interaction was not overcome and higher plasma levels

Table II. *Mean serum levels (± S E.) on the second and fourth day 3 and 8 h after the first of the two daily doses of 500 mg tetracycline. A dose of 400 mg ferrous sulphate was taken either simultaneously with or 2 h after tetracycline*

Number of subjects in each group = 5

	Serum tetracycline (µg/ml)			
	Second day		Fourth day	
Time interval between iron and tetracycline	3 h	8 h	3 h	8 h
Simultaneous intake	1.56 ± 0.27	0.73 ± 0.05	1.40 ± 0.25	0.86 ± 0.19
Iron 2 h after	5.44 ± 0.59	3.76 ± 0.21	4.54 ± 0.64	2.84 ± 0.33
Tetracycline alone	5.46 ± 0.47	4.68 ± 0.60	7.26 ± 0.37	4.82 ± 0.27

of this antibiotic were not reached. On the contrary by the fourth day several subjects had serum tetracycline levels even substantially below 0.6 µg/ml, i.e. M.T.C. When the iron was taken two hours after the tetracycline, adequate plasma levels were maintained, although a slight decrease was noted on the fourth day.

This study was performed with tetracycline chloride in capsules. In the light of our earlier work (5) it seems likely that the precautionary time interval recommended here is also long enough for other tetracycline derivatives. In practice, however a far greater factor of variability is introduced with the iron-containing drugs, of which today there is a broad range of different pharmaceutical forms available. Accordingly it must be stressed that the time interval of two to three hours was found to apply to iron in ordinary sugar-coated tablets.

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Table IV *Concentration of plasma lipids and lipoproteins in women after exclusion of obese persons and persons consuming sex hormones*

Age group	Total lipids (g/l)			Cholesterol (mmol/l)			Triglycerides (mmol/l)			Phospholipids (mmol/l)			Free glycerol (mmol/l)		
	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄
11–20	5.75	0.69	0.14	5.54	0.91	0.19	0.87	0.29	0.06	2.66	0.70	0.15	0.11	0.04	0.008
	0.757	*0.053*	*0.011*	*0.737*	*0.075*	*0.016*	*−0.087*	*0.137*	*0.033*	*0.414*	*0.097*	*0.020*	*−0.978*	*0.145*	*0.030*
21–30	5.41	0.81	0.17	5.63	1.12	0.23	0.75	0.26	0.06	2.53	0.44	0.09	0.11	0.04	0.009
	0.728	*0.066*	*0.014*	*0.742*	*0.088*	*0.018*	*−0.148*	*0.148*	*0.031*	*0.396*	*0.082*	*0.017*	*−0.990*	*0.163*	*0.034*
31–40	6.32	0.97	0.22	6.44	1.18	0.26	0.95	0.40	0.09	2.83	0.62	0.14	0.10	0.04	0.008
	0.796	*0.064*	*0.014*	*0.801*	*0.084*	*0.019*	*−0.063*	*0.197*	*0.044*	*0.440*	*0.106*	*0.024*	*−1.040*	*0.216*	*0.048*
41–50	6.77	1.05	0.23	7.09	1.32	0.29	1.02	0.43	0.09	2.98	0.40	0.09	0.10	0.04	0.009
	0.826	*0.066*	*0.014*	*0.844*	*0.080*	*0.018*	*−0.028*	*0.181*	*0.039*	*0.471*	*0.056*	*0.012*	*−1.018*	*0.198*	*0.043*
51–60	8.02	1.29	0.29	8.32	1.24	0.28	1.10	0.41	0.09	3.28	0.47	0.11	0.11	0.04	0.008
	0.899	*0.070*	*0.016*	*0.915*	*0.067*	*0.015*	*0.007*	*0.183*	*0.041*	*0.511*	*0.064*	*0.014*	*−0.995*	*0.156*	*0.035*
61–70	8.19	1.26	0.27	8.45	1.58	0.34	1.30	0.47	0.10	3.30	0.54	0.12	0.13	0.05	0.010
	0.909	*0.067*	*0.014*	*0.920*	*0.079*	*0.017*	*0.086*	*0.166*	*0.035*	*0.512*	*0.075*	*0.016*	*−0.907*	*0.163*	*0.035*

x̄ Mean value. S.D. = Standard deviation. S.D.x̄ = Standard error of the mean.
Figures in italics are logarithmic values.

Table V *Concentration of plasma lipids and lipoproteins in men after exclusion of obese persons*

Age group	Total lipids (g/l)			Cholesterol (mmol/l)			Triglycerides (mmol/l)			Phospholipids (mmol/l)			Free glycerol (mmol/l)		
	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄	x̄	S.D.	S.D.x̄
11–20	5.36	0.69	0.13	5.01	0.73	0.14	0.88	0.23	0.04	2.14	0.37	0.07	0.09	0.04	0.007
	0.726	*0.057*	*0.011*	*0.696*	*0.064*	*0.012*	*−0.069*	*0.115*	*0.021*	*0.323*	*0.077*	*0.014*	*−1.133*	*0.264*	*0.049*
21–30	5.85	1.20	0.25	5.63	1.12	0.23	1.11	0.36	0.07	2.36	0.41	0.08	0.07	0.03	0.005
	0.759	*0.081*	*0.016*	*0.743*	*0.082*	*0.017*	*0.023*	*0.136*	*0.028*	*0.366*	*0.075*	*0.015*	*−1.153*	*0.146*	*0.030*
31–40	6.29	0.74	0.15	6.28	0.94	0.19	1.15	0.53	0.11	2.75	0.44	0.09	0.10	0.03	0.006
	0.796	*0.052*	*0.010*	*0.793*	*0.065*	*0.013*	*0.023*	*0.180*	*0.036*	*0.434*	*0.070*	*0.014*	*−1.044*	*0.145*	*0.029*
41–50	7.42	1.63	0.31	7.31	1.47	0.28	1.65	0.79	0.15	2.91	0.85	0.16	0.09	0.04	0.008
	0.861	*0.093*	*0.018*	*0.855*	*0.088*	*0.017*	*0.177*	*0.183*	*0.035*	*0.444*	*0.138*	*0.027*	*−1.098*	*0.207*	*0.040*
51–60	7.17	1.16	0.19	7.26	1.15	0.19	1.32	0.62	0.10	2.84	0.60	0.10	0.10	0.04	0.008
	0.850	*0.069*	*0.012*	*0.855*	*0.070*	*0.012*	*0.079*	*0.185*	*0.031*	*0.444*	*0.100*	*0.017*	*−1.028*	*0.147*	*0.024*
61–70	7.04	1.28	0.28	7.00	1.44	0.31	1.35	0.81	0.18	2.79	0.57	0.13	0.10	0.05	0.010
	0.841	*0.079*	*0.017*	*0.836*	*0.091*	*0.020*	*0.065*	*0.240*	*0.052*	*0.437*	*0.094*	*0.021*	*−1.064*	*0.223*	*0.045*

x̄ = Mean value. S.D. = Standard deviation. S.D.x̄ = Standard error of the mean.
Figures in italics are logarithmic values.

Table VIII. High correlation was found between plasma cholesterol and β-lipoproteins and between triglycerides and pre β-lipoproteins, whereas none of the lipid parameters had high correlation to the α-lipoprotein concentration.

DISCUSSION

Frequency distribution

The uniformly good fitness to the normal distribution after logarithmic transformation of the values is noteworthy However due to the rather small coefficients of variation in some of the parameters, the deviation from the normal distribution without logarithmic transformation of the values was not large, and essentially the same statistical conclusions were reached whether using the non-logarithmic or the logarithmic values. Those parameters showing the poorest fitness to the normal distribution without logarithmic transformation of the values were triglycerides and pre-β-lipoproteins. This was also found in respect of triglycerides by Carlson and Lindstedt (7)

Chylomicrons (g/l)			β-lipoproteins (g/l)			Pre-β-lipoproteins (g/l)			α-lipoproteins (g/l)		
$\bar{x}$	S.D.	S.D.$_{\bar{x}}$	$\bar{x}$	S.D.	S.D.$_{\bar{x}}$	$\bar{x}$	S.D.	S.D.$_{\bar{x}}$	$\bar{x}$	S.D.	S.D.$_{\bar{x}}$
0.16	0.11	0.02	3.84	0.95	0.20	0.97	0.40	0.08	3.30	1.17	0.24
−0.892	*0.327*	*0.068*	*0.576*	*0.107*	*0.022*	*−0.050*	*0.177*	*0.037*	*0.493*	*0.151*	*0.032*
0.16	0.14	0.03	3.80	0.78	0.16	0.78	0.46	0.10	3.06	0.87	0.18
−0.859	*0.439*	*0.092*	*0.577*	*0.088*	*0.018*	*−0.170*	*0.242*	*0.050*	*0.468*	*0.129*	*0.027*
0.16	0.10	0.02	4.29	1.23	0.28	0.99	0.54	0.12	3.71	0.92	0.21
−0.889	*0.329*	*0.074*	*0.616*	*0.124*	*0.028*	*−0.093*	*0.325*	*0.073*	*0.556*	*0.111*	*0.025*
0.15	0.09	0.02	4.86	1.22	0.27	1.11	0.49	0.11	3.57	1.10	0.24
−0.833	*0.198*	*0.043*	*0.673*	*0.110*	*0.024*	*0.004*	*0.197*	*0.043*	*0.545*	*0.131*	*0.029*
0.19	0.10	0.02	6.02	1.45	0.32	0.98	0.55	0.12	4.35	1.04	0.23
−0.785	*0.304*	*0.068*	*0.767*	*0.107*	*0.024*	*−0.091*	*0.299*	*0.067*	*0.627*	*0.104*	*0.023*
0.23	0.08	0.02	6.26	1.52	0.32	1.23	0.68	0.14	3.78	0.97	0.21
−0.653	*0.144*	*0.031*	*0.785*	*0.100*	*0.021*	*0.038*	*0.260*	*0.055*	*0.563*	*0.119*	*0.025*

Chylomicrons (g/l)			β-lipoproteins (g/l)			Pre-β-lipoproteins (g/l)			α-lipoproteins (g/l)		
$\bar{x}$	S.D.	S.D.$_{\bar{x}}$	$\bar{x}$	S.D.	S.D.$_{\bar{x}}$	$\bar{x}$	S.D.	S.D.$_{\bar{x}}$	$\bar{x}$	S.D.	S.D.$_{\bar{x}}$
0.14	0.09	0.02	3.47	0.76	0.14	1.00	0.54	0.10	3.13	0.86	0.16
−0.934	*0.337*	*0.063*	*0.530*	*0.100*	*0.019*	*−0.088*	*0.324*	*0.060*	*0.479*	*0.121*	*0.023*
0.18	0.19	0.04	4.00	1.09	0.22	1.16	0.39	0.08	2.87	0.65	0.13
−0.822	*0.313*	*0.064*	*0.589*	*0.109*	*0.022*	*0.038*	*0.159*	*0.032*	*0.447*	*0.106*	*0.022*
0.18	0.10	0.02	4.55	0.85	0.17	1.17	0.59	0.12	2.90	0.69	0.14
−0.840	*0.319*	*0.064*	*0.651*	*0.080*	*0.016*	*0.011*	*0.238*	*0.048*	*0.449*	*0.107*	*0.021*
0.23	0.11	0.02	5.19	1.52	[illegible]	1.81	0.87	0.17	2.84	1.08	0.21
−0.704	*0.271*	*0.052*	*0.699*	*0.121*	*0.023*	*0.213*	*0.201*	*0.039*	*0.434*	*0.165*	*0.032*
0.16	0.08	0.01	5.32	1.08	0.18	1.58	0.77	0.13	2.78	0.89	0.15
−0.830	*0.243*	*0.041*	*0.718*	*0.083*	*0.014*	*0.134*	*0.276*	*0.046*	*0.425*	*0.136*	*0.023*
0.21	0.13	0.03	5.22	1.20	0.26	1.45	1.02	0.22	2.75	0.80	0.18
−0.715	*0.227*	*0.030*	*0.707*	*0.098*	*0.021*	*0.072*	*0.285*	*0.062*	*0.434*	*0.117*	*0.026*

Comparison with other lipoprotein and lipid studies

The present results from quantitative lipoprotein electrophoresis are in very good accordance with results based on ultracentrifugation (17 23 27 30) The results are also directly comparable with those found by a method based on nephelometry (33) Our values in the young age groups are practically identical with the values in the papers mentioned. This seems to confirm the reliability of our method. In the older age groups, where exogenous factors, e.g. dietary habits, in different populations may ha the greatest cumulative influence, our values of β-lipoproteins are generally higher Consequently the levels of these lipoproteins seem to be higher in the Danish population than in the British and American populations re ported above. The levels of α-lipoproteins, how ever are identical with ours. The examination of Polano et al. (30) from the Netherlands shows values for all lipoprotein fractions identical with ours. In conclusion it may be stated that lipoprotein electrophoresis on agarose gel, quantitated according t the directions given in previous papers (10, 11 12) gives results comparable with the ultracentrifugation technique.

In recent investigations of Swedish and Danish populations, the level of plasma cholesterol (7)

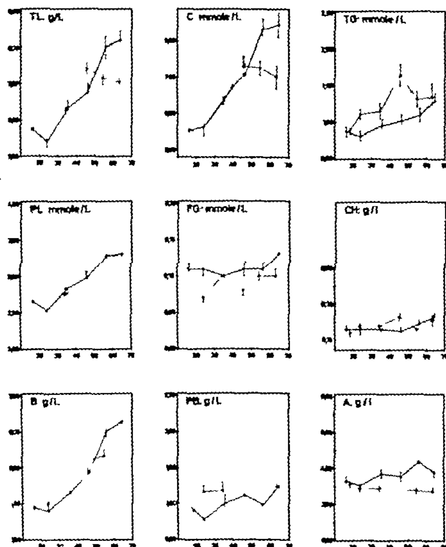


Fig. 1. Mean values with standard errors of the means of plasma lipids and lipoproteins in non-obese persons. x axis = age in years, y-axis = concentrations in units indicated in each coordinate system, ●—● = women, ●- -● = men. Abbreviations as in Table VI.

and triglycerides (7, 8) in different population groups have been examined. Our results show very close accordance with their groups of healthy individuals with identical age and sex patterns. This might be due to the minor difference in living pattern between the urbanized communities in the two Scandinavian countries. Other investigators have examined the values of cholesterol and phospholipids in Danish (20, 25, 31), Norwegian (26) and Swedish (24) populations. Our cholesterol values are generally higher than those of the studies referred to, whereas the phospholipid concentration shows close accordance (20, 24, 31). Part of the explanation of the difference in cholesterol concentrations might be that blood donors were used in two of the studies (25, 26).

Lipid and lipoprotein values related to age and sex

As in the study by Carlson and Lindstedt (7) we found no difference between the sexes in plasma cholesterol concentration in the younger age groups except for higher values in women in the age group 11–20 years. The same pattern was found for β-lipoproteins. From the age of 15 years the cholesterol concentration increased by

0.085 mmol/l (33 mg/l) per year in men up to the age of 50, after which the values declined. The increase for β-lipoproteins in men was 63 mg/l per year. In women the rise in plasma cholesterol concentration continued throughout the age groups, with a total increase of 0.061 mmol/l (24 mg/l) per year for cholesterol and 51 mg/l per year for β-lipoproteins. If the youngest age group is excluded, the increase in cholesterol and β-lipoproteins for men was 30 mg/l and 55 mg/l per year respectively in the age group 21–50 years, and for women 27 mg/l and 61 mg/l, respectively in the age group 21–70 years. The increase in plasma cholesterol with age is in accordance with that of other studies from western countries (1, 19). The age and sex pattern for phospholipids was identical with that of cholesterol and β-lipoproteins, with an increase per year of

Table VI. *Lipid and lipoprotein values in obese and non-obese persons*

		Non-obese Men n = 93, $\bar{x}$ = 49.5 y S.D. = 12.8 y Women n = 42, $\bar{x}$ = 55.6 y S.D. = 14.2 y			Obese Men n = 32, $\bar{x}$ = 49.9 y S.D. = 12.4 y Women n = 24, $\bar{x}$ = 58.3 y S.D. = 13.5 y		
		$\bar{x}$	S.D.$_{\bar{x}}$	Median	$\bar{x}$	S.D.$_{\bar{x}}$	Median
TL	♂	7.03	0.15	6.86	7.44	0.23	7.47
	♀	7.74	0.22	7.74	8.28	0.30	7.79
C	♂	7.04	0.14	6.90	7.16	0.25	6.97
	♀	8.01	0.25	8.13	8.21	0.33	7.89
TG	♂	1.38	0.07	1.19	1.75	0.13	1.61
	♀	1.14	0.07	1.04	1.80	0.17	1.56
PL	♂	2.77	0.07	2.75	3.04	0.14	2.99
	♀	3.30	0.08	3.21	3.22	0.10	3.20
FG	♂	0.09	0.004	0.09	0.10	0.006	0.11
	♀	0.12	0.007	0.11	0.13	0.009	0.13
CH	♂	0.19	0.01	0.17	0.16	0.02	0.16
	♀	0.19	0.02	0.20	0.22	0.02	0.22
B	♂	5.15	0.14	4.96	5.08	0.21	4.93
	♀	5.91	0.24	5.80	6.05	0.23	5.84
PB	♂	1.51	0.09	1.32*	2.08	0.16	2.00*
	♀	1.08	0.10	0.99*	1.78	0.20	1.51
A	♂	2.81	0.09	2.66	2.72	0.11	2.64
	♀	3.78	0.16	3.84	3.22	0.24	3.07

TL = total lipids (g/l), C = cholesterol (mmol/l), TG = triglycerides (mmol/l), PL = phospholipids (mmol/l), FG = free glycerol (mmol/l), CH = chylomicrons (g/l), B = β-lipoproteins (g/l), PB = pre-β-lipoproteins (g/l), A = α-lipoproteins (g/l).

* indicates significant difference between the groups.

Table VII. *Lipid and lipoprotein values in women with and without sex hormone intake (obese persons excluded)*

Abbreviations as in Table VI

	No sex hormone intake n = 100	$\bar{x}$ = 37.5 y	S.D. = 14.8 y	Sex hormone intake n = 26 y	$\bar{x}$ = 36.8 y	S.D. = 14.0 y
	$\bar{x}$	S.D.$_{\bar{x}}$	Median	$\bar{x}$	S.D.$_{\bar{x}}$	Median
TL	6.50	0.13	6.31	6.56	0.21	6.63
C	6.66	0.15	6.52	6.21	0.24	6.20
TG	0.96	0.04	0.93	1.21	0.13	1.15
PL	2.84	0.06	2.78	2.86	0.08	2.92
FG	0.11	0.004	0.10	0.11	0.009	0.10
CH	0.16	0.01	0.17	0.17	0.03	0.17
B	4.60	0.14	4.36	4.32	0.23	4.17
PB	0.98	0.05	0.88	1.17	0.11	1.17
A	3.62	0.11	3.57	3.78	0.19	3.60

0.014 mmol/l (10 mg/l) for men of 11–50 years, after which the values declined a little. In women the increase throughout the age groups was 0.015 mmol/l (9 mg/l) per year.

Triglycerides and pre-β-lipoproteins were found to be significantly higher in men than in women, and with the same sex pattern as that of cholesterol and β-lipoproteins, with a maximum for men in the age group 41–50 years, after which age the level declined. The decline in triglycerides first described by Carlson and Lindstedt (7) thus seems to be confirmed and to be the result of a decline in pre-β-lipoproteins, as no variation was found in the concentration of chylomicrons. α-lipoproteins showed a clear sex difference with higher

Table VIII. *Correlation coefficients between the lipid and lipoprotein parameters*

Abbreviations as in Table VI
All r-values are significantly different from zero

Compounds	Correlation coefficient (r)
C–B	0.900
C–PB	0.309
C–A	0.153
TG–B	0.338
TG–PB	0.822
TG–A	−0.184
PL–B	0.637
PL–PB	0.278
PL–A	0.294

values in women, whereas no age-dependent variation could be demonstrated.

Influence of obesity

Significantly higher levels of plasma triglycerides due to an elevation of the triglyceride rich pre β-lipoproteins were found among obese persons of both sexes (Table VI) Elevated serum triglyceride concentration in obese subjects has been reported by several authors (2, 5 7 8 34) whereas reports of lipoprotein elevations are more scarce (22) Examination of a possible correlation between serum cholesterol and obesity has given diverging results, as summarized by Carlson and Lindstedt (7) In this and in Lindholm s study (24) we found no such correlation The same was seen in the cholesterol-rich β-lipoprotein fraction. Phospholipids were somewhat higher among obese men than among non-obese. The difference is, however not significant (p_{ss} = 0.061) This is in accordance with Lindholm (24) A significantly lower α-lipoprotein concentration in obese women than in non-obese as found in our investigation has, to our knowledge, not been described earlier and needs confirmation for general acceptance. The explanation of this observation is obscure at the present time. The possibility that our figures could be due to chance is rather remote (p_{ss} = 0.016 Wilcoxon)

Effect of sex hormone intake

Due to the increasing use of oral contraceptive therapy careful attention has been paid to its influence on the general metabolism. Increased levels of plasma lipids and lipoproteins in women taking these drugs have been observed by several authors, who have found elevation of plasma cholesterol, triglycerides, phospholipids, and low density lipoproteins (4 9 16, 21 35) As the main effect on the lipid metabolism by these drugs is considered to be due to their estrogen component (6) our sex hormone treated group consists of women taking contraceptive drugs and women on postmenopausal estrogen substitution therapy Though a tendency to higher triglyceride and pre β-lipoprotein levels was found in our study in the hormone treated group, none of these differences were significant ($0.05 < p < 0.10$) The inhomogeneity of drugs used in the present study could be one of the reasons for our failure to demonstrate a significant plasma-lipid-increasing effect.

Abnormal lipoprotein types

The occurrence of a double pre-β-lipoprotein fraction has been reported by other workers (18 29) especially in hyperlipidaemic states and in persons treated with lipid-decreasing drugs, but also in some normal subjects. Neither the frequency of this abnormality nor its eventual clinical significance are known. In our investigation the frequency seems to be equal in the two sexes (approx. 7%) and not related to age, obesity or sex hormone intake.

Correlation between lipid and lipoprotein parameters

The high correlation coefficients between plasma cholesterol and β-lipoproteins, and between plasma triglycerides and pre-β-lipoproteins (Table VIII) indicates that the plasma cholesterol and triglyceride concentrations provide a fairly good expression of the plasma β- and pre-β-lipoprotein concentrations, respectively in cases when the chylomicrons are not elevated.

However hypertriglyceridaemia may equally be due to hyper pre-β-lipoproteinaemia or to hyperchylomicronaemia. Consequently the correlation coefficient between plasma triglycerides and pre β-lipoproteins may give a false impression of this relation, as it is only correct when insignificant amounts of chylomicrons are present in the plasma. When the lipoprotein pattern is otherwise abnormal, e.g. the abnormality with "broad beta band" described by Fredrickson et al. (15) this correlation coefficient is without meaning. Due to this and to the increasing interest in plasma lipid and lipoprotein determinations brought about by the relation between these parameters and coronary atherosclerosis, the need for quantitative lipoprotein determination is obvious. The method which has been used by us, and the validity of which has been confirmed by this study seems to fulfil this need being relatively simple to perform and not necessitating complicated and expensive laboratory equipment.

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LB-46, A NEW β-ADRENERGIC RECEPTOR BLOCKING AGENT IN CARDIAC ARRHYTHMIAS

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Abstract. A new β-adrenergic receptor blocking agent, LB-46 Visken, Sandoz (DL 4-(2-hydroxy-3-isopropylamino-propoxy)indole) has been tried in various arrhythmias in 22 patients. It was effective in reducing the heart rate in sinus tachycardia, rapid atrial fibrillation and some cases of supraventricular and ventricular tachycardia. LB-46 had no serious side-effects and did not precipitate heart failure in any of our patients. It has an intrinsic sympathomimetic activity. LB-46 was given to two pregnant patients in their last trimester with no adverse effect. The mechanism for the antiarrhythmic properties of β-adrenergic receptor blocking agents is discussed.

The usefulness of β-blocking agents in different kinds of arrhythmias is well established. Their mode of action, however, is not fully agreed upon. Some of their properties seem to be due to a membrane-stabilizing or quinidine-like effect, while others are a result of the β-blocking mechanism.

LB-46 Visken, Sandoz, is a new potent β-blocking agent. The doses necessary are smaller than those for pronethalol, propranolol (Inderal®), alprenolol (Aptin®) and ICI 50172 (Practolol).

The effects of LB-46, (DL 4-(2-hydroxy-3-isopropylaminopropoxy)indole), in vivo and in vitro have been studied in different animals and it is concluded that LB-46 is a potent β-receptor blocking agent, its potency being 5–30 times that of propranolol (13). Hill and Turner (6) have investigated the effect of LB-46 in two doses (0.5 mg and 2 mg orally) and of propranolol (20 mg orally) on exercise-induced and isoprenaline-induced tachycardia in normal volunteers and found the potency of LB-46 to be 20–40 times that of propranolol.

LB-46 had not the negative chronotropic effect on resting heart rate seen with propranolol, and the authors feel that this may be due to an intrinsic sympathomimetic activity of LB-46.

We have tried LB-46 in a group of 22 patients with different kinds of arrhythmias as described below.

MATERIAL AND METHODS

The plasma concentrations of LB-46 were determined by fluorimetric method as described by Pacha (12).

Sinus tachycardia

Four patients had sinus tachycardia with heart rates between 100 and 135. One of these patients had thyrotoxicosis, one arterial hypertension, one nephrotic syndrome, and one young woman sinus tachycardia of unknown origin.

LB-46 was administered i. in dose of 0.4 mg. The patients were connected to an oscilloscope and ECG was recorded after 1 2, 5 10, 15 20, 30, 45 and 60 min (Fig. 1). Heart rate was measured from the ECG. None of the patients suffered any adverse effect, and no arrhythmias or extrasystoles were seen on the oscilloscope.

LB-46 acted rapidly. An effect on the heart rate was seen after 1 min and full effect after 2 or 5 min. The heart rate remained at the same level during the rest of the observation period of 30 to 45 min. The patient with thyrotoxicosis was also given the double dose, 0.8 mg i. which caused further decrease in heart rate by 10 beats/min.

Atrial fibrillation

LB-46 was given to six patients (cases 1–6) with atrial fibrillation and rapid ventricular rate (Table I).

Case 6. Female, born in 1946, developed atrial fibrillation in the last trimester of pregnancy. She had heart rate of 140 on digitoxin, 0.1 mg day with reduction to 120–130 on 0.2 mg digitoxin daily and increasing dyspnoea, venous congestion and liver enlargement. Treatment with LB-46, 5 mg 3 daily reduced the heart rate to 90–100 and markedly improved the patient's condition. The foetal heart

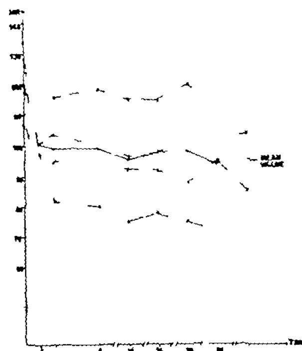


Fig. 1. Effect of LB-46 (0.4 mg i.v.) on sinus tachycardia (4 cases).

rate was within normal range and the patient delivered a healthy boy. Plasma concentrations of LB-46 in blood samples from mother and child were 18.4 and 11.7 ng/ml, respectively.

Supraventricular tachycardias and arrhythmias

Case 7. Male, born in 1912, had paroxysmal A-V-junctional tachycardia of increasing frequency and duration (HR 160). Quinidine, digitalis, alprenolol (Aptin), antazoline and diphenylhydantoin in adequate doses had no effect, nor had LB-46 30 mg daily. Sinoatrial block was then recorded and thought to initiate the attacks of tachycardia and, after implantation of an on-demand (Ectocor) pacemaker the attacks subsided.

Case 8. Female, born in 1948, had paroxysmal atrial tachycardia without and with block with a ventricular rate of 160. Adequate doses of digitalis, diphenylhydantoin, quinidine, propranolol (Inderal) and alprenolol (Aptin) were without effect. On follow-up 3 months later LB-46 15 *mg daily (plasma concentrations 20–30 ng/ml)* had reduced both the number of attacks and the heart rate during attacks to 130–140.

Case 9. Female, born in 1947 had coronary sinus rhythm with a resting heart rate of 130–150 and attacks of retrosternal pain, numbness of the left arm and dysphasia on one occasion. The patient was tested on a bicycle ergometer (Fig. 2). LB-46 i.v. and orally reduced the heart rate, but treatment had to be discontinued because of dizziness and nausea. Atropine, reserpine, dihydroergotamine, prednisone, digitalis and electroconversion had been tried before.

Case 10. Female born in 1945 had had occasional attacks of paroxysmal tachycardia since the age of 15. During pregnancy her complaints of palpitations, dizziness, dyspnoea, especially after physical exertion and meals (HR 180–200), increased and ECG showed chaotic atrial mechanism (13). Digitalis was without effect and treatment with LB-46 15 mg daily was started in the sixth month of pregnancy. *The attacks subsided,* but on

Table I. *Effect of LB-46 in atrial fibrillation*

Case no.	Born in	Sex	Disease	Previous treatment	Symptoms	Effect of LB-46
1	1917		Thyrotoxicosis	Carbimazol	Thyrotoxicosis, congestive heart failure	0.4 mg i. HR 150→115
2	1905	♂	Thyrotoxicosis	Carbimazol, digitalis	Thyrotoxicosis, congestive heart failure, pulmonary oedema	0.4 mg i. HR 100→72
3	1906		Rheumatic heart disease	Mitral commissurotomy, aortic ball valve prosthesis, electroconversion, digitalis	Palpitations, dyspnoea	2.5 mg ×3 p.o. HR 140→90
4	1919		Rheumatic heart disease	Mitral ball valve prosthesis, electroconversion, digitalis	Palpitations, dyspnoea	0.4 mg i. HR 130→70
5	1925	♀	Rheumatic heart disease	Mitral commissurotomy, aortic ball valve prosthesis, electroconversion, digitalis	Palpitations on exertion	0.4 mg See Fig. 5
6	1946	♀	Rheumatic heart disease	Mitral commissurotomy, mitral annuloplasty, digitalis	Congestive heart failure, palpitations	5 mg ×3 p.o. HR 125→95 See text

ferent occasions she had isorhythmic atrioventricular dissociation, wandering atrial pacemaker and multiple ectopic atrial beats. Delivery was uncomplicated with the use of vacuum extractor and no foetal bradycardia was observed during pregnancy or thereafter. Plasma concentration of LB-46 was 40.0 ng/ml in maternal and 1..5 ng/ml in foetal blood.

Bigeminy

Two young men with chronic myocarditis had ventricular extrasystoles mostly as bigeminy which increased during physical exertion. No effect of LB-46 was observed, but diphenylhydantoin had good effect in one.

Ventricular tachycardia

Three patients with paroxysmal ventricular tachycardia, in two due to coronary heart disease were treated with LB-46.

Case 11. Female born in 1902. Digitalis, quinidine, procainamide, LB-46 (22.5 mg daily), plasma concentration (30–60 ng/ml), were without effect, but diphenylhydantoin eliminated the attacks.

Case 12. Male, born in 1924, had anteroseptal infarction in 1968 and since then severe attacks of ventricular tachycardia with chest pain and syncope. After transfer to our Department in August 1969 he had to be continuously treated with lignocaine drip (2–4 mg/min) combined with bolus doses during attacks, which led to cerebral confusion on two occasions.

Digitalis, procainamide, quinidine, diphenylhydantoin, ICI 50172 (100 mg × 2), cardiac pacing up to a frequency of 150 (HR during attacks 180–200), were ineffective, whereas LB-46 10 mg × 3 suppressed the ventricular extrasystoles, but did not prevent the attacks. In Oct. 1969 left-sided sympathectomy was performed, the attacks subsided, but marked ventricular extrasystoles, which still persisted, disappeared on LB-46 15 mg daily. One year after he was well and had not been rehospitalized.

Case 13. Female, born in 1914. Since 1951 she has had attacks of paroxysmal ventricular tachycardia accompanied by chest pain, syncope, diarrhoea and polyuria with increasing frequency. She was hospitalized and defibrillated several times from 1968 onwards. No effect of propranolol in small doses (80 mg/d.), diphenylhydantoin, procainamide (6 g/d.), quinidine or ICI 50172 400 mg daily. LB-46 in increasing doses up to 10 mg 3 times daily completely prevented the attacks, and half a year later she had no extrasystoles. Her dysrhythmia was probably due to coronary artery disease, since exercise ECG showed ischaemic changes.

Patients with implanted pacemakers

Case 14. Female, born in 1909 had on-demand pacemaker because of sinoatrial block with paroxysms of rapid, atrial fibrillation. Digitoxin 0.1–0.2 mg daily produced nausea without adequate suppression of the heart rate. LB-46 (15 mg/d.) reduced the heart rate during attacks from 130 to 90.

Case 15. Female, born in 1907, had paroxysmal tachycardia from 1965, the attacks increasing in duration from 1968. She had tried digitalis, quinidine, procainamide and

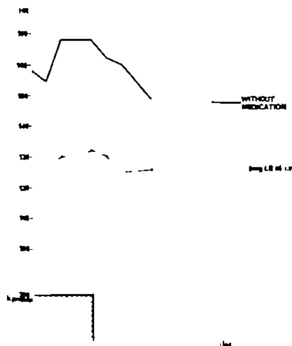


Fig. 2. Exercise test on a bicycle ergometer before and after LB-46. On both occasions the patient had to stop working because of dyspnoea and exhaustion (case 9).

lignocaine drip without effect, but alprenolol (Aptin) given in combination with Lanatosid-C produced total A-V block with a heart rate of 33. In our Department pacing with a rate of 110 was ineffective and an on-demand pacemaker was implanted. She still had attacks of atrial flutter (and fibrillation) with HR 180–200, which did not respond to electroconversion, and her congestive heart failure increased. LB-46 5 mg 3 times daily was given, and on the same day fixed rate pacemaker rhythm with no P waves was recorded (plasma concentration 100 ng/ml). After one month she was still free from attacks.

Case 16. Male, born in 1916. An atrial triggered P synchronous pacemaker was implanted because of total A-V block. The patient experienced irregular heart action and dyspnoea on exertion. Testing on a bicycle ergometer before and after LB-46 proved the patient feeling of arrhythmia to be caused by a pacemaker 2 : 1 block when the heart rate had reached 126, a normal function of the P-synchronous pacemaker (Figs. 3 and 4). LB-46 15 mg daily led to subjective improvement.

DISCUSSION

The antiarrhythmic action of β-blocking agents was first shown with DCI (dichloroisoproterenol) 1959 (16). It was soon established (10) that the antiarrhythmic effect was due to two different mechanisms, a β-blocking effect and a membrane

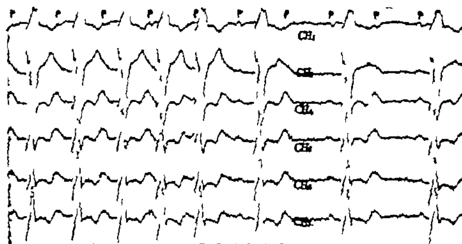


Fig. 3. Development of pacemaker 2 : 1 block, a normal function of the P-synchronous pacemaker when the atrial rate reaches 120–130 (case 16).

effect (called "quinidine like", "local anaesthetic" or "membrane-stabilizing" effect). The β-blocking property is due to the levoform, as much higher doses of the dextroform (40–100 times) (7, 9) are needed to produce β-blockade, whereas the membrane effect is similar for the two isomers.

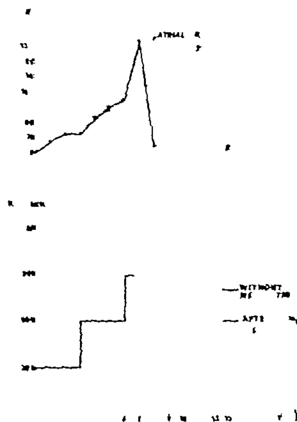


Fig. 4. Effect of LB-46 on development of pacemaker 2 : 1 block. After LB-46 a greater external work load is needed to produce the block (case 16).

β-adrenergic blocking action may be assessed quantitatively by reversal of isoproterenol-induced tachycardia and increased force of myocardial contraction or reversal of isoproterenol-induced peripheral vasodilatation (1). The effects of propranolol on cardiac conduction have been studied by His bundle ECG by Berkowitz et al. (2). A-V conduction was significantly prolonged and intraventricular conduction unchanged when the heart rate was kept constant by atrial pacing; this effect can be explained on the basis of β-blockade.

Membrane effect. Experimentally propranolol has been shown to reduce the duration of the action potential in atrial muscle and Purkinje fibres (4) due to shortening of the early phases of repolarization. The refractory period of the Purkinje fibre was also reduced. The tissues showed a reduced ability to be excited by every stimulus at high frequencies.

The velocity of upstroke of membrane potential and the magnitude of overshoot are reduced by propranolol, an effect similar, but not identical, to that produced by quinidine and some local anaesthetics. In a recent study Kübler and Sowton (8) have shown that the pacing threshold is increased by propranolol.

Sinus tachycardia. β-receptor blocking agents are the only drugs effective in promptly controlling sinus tachycardia. This may be necessary in the

acute stage of thyrotoxicosis, in sinus tachycardia due to anxiety states and in drug-induced sinus tachycardia due, for instance, to hydralazine. The effect is due to β-blockade.

Atrial fibrillation. The prolongation of A-V conduction leads to a decrease in ventricular rate. Six of our patients had atrial fibrillation due to either thyrotoxicosis or organic heart disease. We used a β-receptor blocking agent only when high doses of digitalis did not control the heart rate. Five of the patients with congestive heart failure were improved by lowering the heart rate with LB-46. The sixth patient had no clinical manifestation of heart failure, but an enlarged heart. The heart size was unchanged after 6 months on LB-46.

The heart rate in atrial fibrillation during exercise is also reduced after β-receptor blocking agents (5); this was found in case 5 (Fig. 5). Reversal to sinus rhythm occasionally occurs, especially if the atrial fibrillation is of recent onset (17).

Supraventricular tachycardia and arrhythmias β-blockade seems to be of particular value in tachycardias precipitated by exercise or emotion and in tachycardias in the Wolff Parkinson-White syndrome (4). Reciprocating tachycardia also responds well to propranolol (3). In one patient with a rapid A V junctional tachycardia due to a sinoatrial block, LB-46 had no effect on the heart rate or number of attacks.

In the other three patients with different types of tachycardia LB-46 reduced the heart rate in all, but did not restore sinus rhythm. One patient had to discontinue treatment because of side-effects.

Bigeminy β-receptor blocking agents are very effective in suppressing ectopic beats due to digitalis intoxication, and this effect is a prominent with the dextroisomer as with the racemic form (11). However Sowton (15) reports that practolol, which has practically no membrane effect but purely a β-blocking action, is also effective in suppressing ectopic beats. These results indicate that both actions are of clinical importance. None of our patients had digitalis intoxication, but LB-46 did not suppress ventricular ectopic beats in two young patients with chronic myocarditis.

Ventricular tachycardia. The effect of LB-46 was studied in three cases of resistent ventricular tachycardia. It was wholly successful in one, partly successful in another and without effect in the third case. This illustrates the therapeutic difficulties often encountered in these arrhythmias, in which only empirical trial and error lead to the goal.

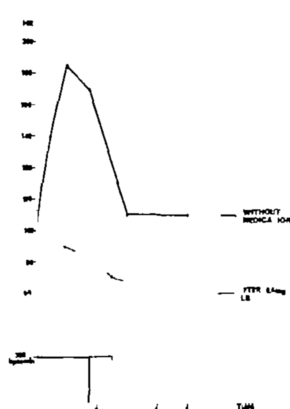


Fig. 5 Effect of 0.4 mg LB-46 on heart rate in patient with atrial fibrillation tested on bicycle ergometer (case 5).

Patients with implanted pacemakers. In two patients LB-46 was used to reduce the heart rate in atrial fibrillation and atrial flutter after pacemaker implantation. Both patients had sinoatrial block alternating with tachycardia. In the third patient with P-synchronous atrial triggered pacemaker LB-46 reduced the exercise-induced tachycardia, and thereby the pacemaker 2 1 block occurring at higher heart rates.

Pregnant patients. In two pregnant patients in their last trimester whose heart rates were not adequately controlled with digitalis, we decided to give LB-46. We have found no reports in the literature on the use of β-adrenergic blocking agents in pregnancy No adverse effect of LB-46 and no foetal bradycardia were noted.

Side-effects. In one patient treatment had to be stopped because of dizziness and nausea. Two

other patients complained of slight dyspepsia, which did not necessitate discontinuation of the drug. Serious side-effects like hypotension, severe bradycardia and congestive heart failure were not observed. The absence of these side-effects may be due to the intrinsic sympathomimetic activity of LB-46

CONCLUSION

β-adrenergic blocking agents have proved useful in different cardiac arrhythmias. Their effects are partly due to β-blockade and partly to a membrane effect and are as yet not wholly understood.

β-adrenergic blocking agents are not a homogenous group of substances, and there are specific differences between them. Failure of one β-adrenergic blocking agent to influence an arrhythmia does therefore not necessarily mean that others may not be effective, as shown in some of our patients. Pregnancy is a contraindication for the use of most new drugs. We saw no adverse effect of LB-46 in two pregnant women in their last trimester and the plasma concentrations were lower in foetal than in maternal blood.

ACKNOWLEDGEMENT

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VASCULAR RESISTANCE IN HYPOTHERMICALLY PERFUSED KIDNEYS FOLLOWING ONE HOUR OF WARM ISCHAEMIA

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Abstract. The vascular resistance in hypothermically perfused rabbit kidneys following one hour of warm ischaemia has been investigated in 45 experiments. As perfusate medium was used 5% low molecular weight dextran in balanced salt solution (TIS-U-SOL) to which 5 mg% papaverine was added. One hour of warm ischaemia following death (cardiac arrest) either by suffocation or bleeding, and with an agonal phase from 3 to 15 min, increased the vascular resistance about three times compared to group with only short period of warm ischaemia. Clamping of the renal artery for one hour revealed similar increase in the vascular resistance, while simultaneous clamping of the renal artery and vein only increased the vascular resistance by about one-third compared to the group with short period of warm ischaemia. Heparinization of the animals before one-hour ischaemia period prevented pronounced increase in the vascular resistance during the first 10 to 20 min of perfusion.

In an earlier work (6) it has been demonstrated that the vascular resistance at the start of hypothermic perfusion of rat kidneys after 7 min of warm ischaemia was significantly reduced if the animals were heparinized before removal of the kidney and papaverine or lidocaine was added to the perfusate medium.

If cardiac arrest is used as death criterion, the use of necro-kidneys in human transplantation means that the time from death of the donor until the start of the hypothermic perfusion of the kidney is often considerably longer than 7 min and, furthermore, hypotension of varying degree and duration may have been present before death.

Renal vasoconstriction during shock has been described in different reports (1 2, 4 5 8, 9) and the development of increased vascular resistance in the agonal phase has been demonstrated by Belzer et al. in experiments with hypothermic perfusions of pig kidneys (1). These workers also demonstrated that human kidneys with high vascular resistance often failed to function after transplantation using continuous hypothermic plasma perfusion as preservation method These results, which are in agreement with the findings of other workers (3 7), indicate that a common cause of posttransplantation renal failure is persistent increased vascular resistance.

Changes in the vascular resistance in the period from arrest of the renal circulation until the start of the hypothermic perfusion are only poorly elucidated. About one hour is generally considered to be the longest acceptable period of warm ischaemia in human donor kidneys, and it is therefore the purpose of this study to investigate the influence of a one-hour ischaemic period on the vascular resistance.

MATERIAL AND METHODS

The experiments were performed in perfusion apparatus which allowed hypothermic perfusion with continuous control of arterial pressure, flow rate and temperature. A detailed description of the apparatus and the perfusion technique has been given previously (6) The only exceptions were that the perfusate medium was filtered through filter (Millipore SCWP 8 μ) placed in the perfusion circuit, that the volume of the recirculating perfusate was increased from 20 to 50 ml, and that about 50 to 100 ml perfusate was used in order to flush the kidneys free from blood before the perfusate medium was allowed to recirculate in the system.

The material included 45 rabbit kidney perfusions. The animals (White Danish Landrace) were anaesthetized by i. administration of nembutalum NFN 25–30 mg kg b.wt. supplied with N_2O and oxygen by mask. At the same time 10% Mannitol 4–5 ml/kg b. t. and isotonic NaCl 4–5 ml/kg b wt. were given. Then catheter (Bardic 2123) was placed intraarterially in the ear of the rabbit and the BP was continuously measured during the surgical procedure by means of pressure transducer (Statham type P23H) connected to BP recorder (Ellab Type

Table I *The arterial pressure in the six groups of rabbit kidney perfusions at various times during the perfusion (mean values and S.D.)*

Group no.	Arterial pressure (mmHg) 1 min.	5 min	10 min	20 min	30 min	40 min	50 min
1	64±21	46±13	39±9	34±5	32±5	31±3	31±3
2	111±17	111±14	108±12	109±15	110±15	111±16	111±17
3	100±19	115±20	113±19	112±15	112±14	112±13	110±11
4	148±39	129±23	113±26	109±29	108±31	109±34	110±34
5	73±19	63±15	57±11	51±9	51±9	50±9	49±9
6	188±69	134±46	85±18	71±20	68±20	66±21	65±21

MCBCMD. N experiments with fall in the mean BP below 70 mmHg before clamping the renal artery wer used in the present material.

The abdomen was opened and, in experiments where clamping of the renal vessels was performed before the start of the ischaemic period (groups 1 4, 5 and 6), a careful ("no touch technique") separation of the vessels was made. In groups 2 and 3 no dissection on the renal pedicle was made before one hour after death.

All the kidneys were perfused in the apparatus with 5% low molecular weight dextran in balanced salt solution (TIS-U-SOL) to which papaverini sulphas NFN was added to concentration of 5 mg%. The flow rate was adjusted on the basis of the kidney weight to be 0.5 ml/g kidney min in all experiments. The flow rate was controlled during the perfusion, and in all experiments the variations ere less than ±5%. The period from removal of the kidney until the start of the perfusion was 7 min. After 10 min perfusion the temperature in the kidneys was 13°C with only small variations and remained constant during the rest of the perfusion. After 50 min perfusion the kidneys were fixed in buffered formalin. Paraffin wax sections were stained with iron haematoxylin, van Gieson's and Lendrum's fibrin stains.

In this paper the period of warm ischaemia was defined as the interval between cardiac arrest, or clamping of the renal artery and the start of the hypothermic perfusion.

The experimental conditions aried before removal of the kidneys and the material as divided into six groups.

Group 1 After separation and clamping of the renal vessels, the kidneys were removed immediately. The animals were heparinized 10 min before clamping (500 I.U. heparinum NFN kg b.wt.). Five experiments. Period of warm ischaemia 7 min.

Group 2 After opening of the abdomen the animals were killed by suffocation. A period from 5 to 15 min passed before cardiac arrest was noted. The kidneys were removed 60 min after cardiac arrest. The animals were heparinized 10 min before suffocation. Five experiments. Period of warm ischaemia 67 min.

Group 3 After opening of the abdomen the animals were killed by bleeding (cutting the aorta). A period from 3 to 10 min passed before cardiac arrest was noted. The kidneys were removed 60 min after cardiac arrest. The animals were heparinized 10 min before bleeding. Five experiments. Period of warm ischaemia 67 min.

Group 4 After separation of the vessels the renal artery was clamped. The kidneys were removed 60 min later. The animals were heparinized 10 min before clamping of the artery. Ten experiments. Period of warm ischaemia 67 min.

Group 5 The same conditions as in group 4 with the exception that both the renal artery and vein ere clamped at the same time. Ten experiments.

Group 6 The same conditions as in group 5 with the exception that the animals were not heparinized before clamping of the vessels. Ten experiments.

RESULTS

Table I shows the changes of the arterial pressures during the perfusions in the six groups.

Group 1 showed the lowest values. With the exception of the pressure in group 5 after 1 min perfusion, the pressure during the whole period was significantly lower ($p < 0.01$) compared to all other values in the different groups.

Groups 2 and 3 showed nearly identical values. After 10 min perfusion, furthermore the values were nearly identical to those of group 4. During the first 20 min perfusion the pressure was lower than in group 4 ($0.02 < p < 0.025$ after 1 min, $0.05 < p < 0.10$ after 5 min). The arterial pressure was about three times higher than in group 1 during most of the perfusion.

Group 4 showed a pressure about three times higher than group 1 during most of the perfusion.

Group 5 showed a pressure about one-third higher than group 1 ($p < 0.01$) and about half that of groups 2, 3 and 4 ($p < 0.01$).

Group 6 showed a significantly higher pressure during the first 20 min perfusion as compared to group 5 ($p < 0.01$). During the rest of the perfusion the mean values were still higher but not significant on the 0.01 level ($0.025 < p < 0.05$).

Histological examination of the kidneys after

50 min perfusion showed no pathological changes in glomeruli, tubules or interstitial tissue apart from a slight vacuolization of the tubular epithelium in two cases. Differences were noted concerning the content of blood in the vascular bed. Groups 1 and 3 were completely free from blood, while groups 2 and 4 had some and groups 5 and 6 varying quantities, but often copious blood retained in the vascular system of the papillae.

DISCUSSION

The experiments show that the vascular resistance in hypothermically perfused rabbit kidneys has increased about three times if a one-hour ischaemic period is *added after the death of the animals* (groups 1, 2 and 3). This was the case even with papaverine in the perfusate medium and after preceding heparinization of the animals. Death following suffocation or due to bleeding showed no difference in the vascular resistance under these conditions (Fig. 1).

It has been demonstrated in experiments with hypothermic perfusions of pig kidneys that increased vascular resistance often develops during the agonal phase (1) and therefore we decided to investigate the vascular resistance in kidneys with a sudden fall in the arterial pressure (clamping of the renal artery) followed by one hour of

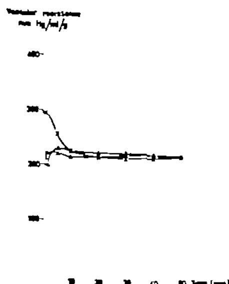


Fig. 1. Changes in the vascular resistance during hypothermic perfusion of rabbit kidneys. Mean values. ▲—▲ group 2; △—△, group 3; — group 4.

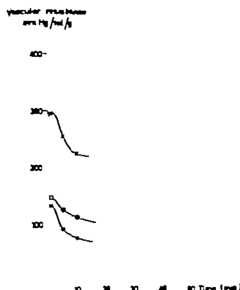


Fig. 2. Changes in the vascular resistance during hypothermic perfusion of rabbit kidneys. Mean values. ●—● group 1; — group 4; ○—○ group 5.

warm ischaemia. The experiments showed that the vascular resistance in this group (group 4) during the last 40 min of the perfusion was nearly identical to the vascular resistance in groups 2 and 3, in which an agonal phase was added before cardiac arrest (Fig. 1).

In groups 2 and 3 the vascular resistance was unchanged from the beginning to the end of the perfusion. In group 4 it was higher at the start and then decreased to stable values after 10 to 20 min. Since this was the case also in the other groups, in which the renal pedicle was manipulated in connection with clamping of the renal vessels before death, the higher vascular resistance at the beginning of the perfusion may be due to stimulation of renal vasoconstrictors in connection with clamping of the vessels. This was omitted in groups 2 and 3, in which surgical manipulation on the renal pedicle was not started before one hour after death.

The kidneys in groups 2, 3 and 4 all turned pale and slightly cyanotic during the ischaemic period. The consistency was reduced and some of the kidneys were slightly wrinkled on the surface. This shows that the volume of the organ diminished during the ischaemic period, probably due to a generalized vasoconstriction. Therefore the vascular resistance was determined in a group

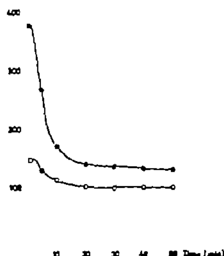


Fig 3 Changes in the vascular resistance during hypothermic perfusion of rabbit kidneys. Mean values. O—O, group 5 ●—● group 6.

in which both the renal artery and vein were clamped at the same time preventing the blood from leaving the vascular bed and collapse of the vessels. These kidneys remained unchanged in consistency and were pronouncedly cyanotic. The vascular resistance in the following hypothermic perfusion showed a marked reduction compared to group 4 and a slight but significant increase compared to group 1 (Fig. 2)

The probability of incipient intravascular coagulation already after 7 min arrest of renal circulation has been demonstrated earlier (6) In experiments with rat kidney perfusions a lower pressure at the start of the perfusion was found if the animals were heparinized before clamping of the renal artery However no difference was found after about 10 min perfusion in kidneys from heparinized and non-heparinized animals. The experiments in this study revealed a more pronounced difference in the vascular resistance in kidneys from heparinized and non-heparinized animals although the difference diminished greatly during continued perfusion (Fig. 3)

Histological examination shows that, even after perfusion with a quantity of perfusate corresponding to 25 ml/g kidney weight, it is difficult to remove the blood from all parts of the vascular bed when a one-hour warm ischaemic period precedes the perfusion.

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COMPARISON BETWEEN ALPRENOLOL AND CHLORTHALIDONE AS ANTIHYPERTENSIVE AGENTS

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Abstract. A double-blind cross-over study comparing alprenolol 100 mg × 4 with chlorthalidone 50 mg × 1 has been performed on 40 subjects. Throughout the study 1.5 g potassium chloride was given daily. In the dosages given both alprenolol and chlorthalidone caused a significant reduction of the BP. Chlorthalidone reduced the BP significantly more than alprenolol. Follow-up studies showed that the effect of alprenolol on the BP increased when the dosage was doubled. In spite of the potassium substitution chlorthalidone reduced serum potassium significantly while no change was found with alprenolol. There was a significant increase in serum uric acid with both alprenolol and chlorthalidone, being significantly more pronounced with chlorthalidone. Subjective side-effects were few and transient both with alprenolol and chlorthalidone.

Population studies have shown that even rather mild hypertension is an important risk factor for coronary heart disease (9, 16). Clinical studies have shown that treatment of asymptomatic hypertension may reduce mortality and morbidity from stroke (5, 20). There are many antihypertensive drugs in clinical use, but the common occurrence of side-effects has made the search for new antihypertensive agents with fewer side-effects urgent. During recent years β-adrenergic blocking agents have been used in arterial hypertension and may be a valuable therapeutic contribution. Their effect is probably due mainly to a reduction of cardiac output (2, 8, 14).

β-adrenergic blocking agents, such as propranolol (12, 22) and alprenolol (4, 19), have caused a significant reduction in arterial BP in hypertensive patients compared to placebo. In the evaluation of a new antihypertensive drug it is also important to compare its effect to that of the conventional medication. In Sweden saluretics are frequently used in mild hypertension, and in the present study a comparison was made between alprenolol (Aptin®) (1) and the saluretic drug chlorthalidone (Hygroton®) (17).

MATERIAL

The subjects of the study were recruited from a population study performed on about 1 500 women aged 38–60 in Göteborg, Sweden, in 1968 and 1969 (3). There were 95 women with a systolic BP ≥ 160 mmHg and diastolic BP > 95 mmHg, who were not on antihypertensive treatment at the time of the population study. Of this number 52 were willing to participate. No other selection was made. These 52 were treated with placebo for one month. After this placebo period 41 had a systolic BP ≥ 160 mmHg and/or diastolic BP > 95 mmHg. These 41 women were given randomized treatment with alprenolol or chlorthalidone. One woman was hospitalized during the treatment period and withdrawn from the study. She was then on chlorthalidone. Pulmonary infarction was considered to be the probable diagnosis and probably non-related to the antihypertensive treatment. Thus 40 women completed the trial. The results of this study refer to these 40 women, aged 38–60 with a mean age of 52 years. None of them had a history or signs of cardiac or renal insufficiency. Heart X-ray was performed on 38 subjects and in all of them the heart volume was found to be < 500 cm³/m² BSA. In one woman it exceeded 450 and in another seven women 400 ml/m² BSA. All subjects had serum creatinine < 1.2 mg/100 ml. None had severe eyeground changes. Keith–Wagener–Barker grade I–II was found in 29 subjects, while no hypertensive changes were found in 11.

METHODS

Cross-over study

Fig. 1 shows the design of the study. The periods with placebo were single-blind, while those with alprenolol and chlorthalidone were double-blind, using a cross-over technique. The periods with alprenolol and chlorthalidone each lasted 3 months, the total time for the study includ-

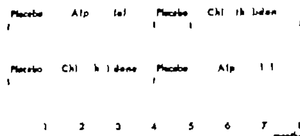


Fig 1 Design of the study

ing the two placebo periods being 8 months. All the participants started and finished the study at about the same time.

BP was measured every two or four weeks as shown in Fig. 1 A venous blood sample was drawn at the end of the placebo and treatment periods. The BP was measured in the sitting, lying and standing positions after about 10 min rest. All the BP measurements were performed by the author Readings were taken from the right arm, using a 23 12 cm cuff ith nylon-hooklet binding, with a mercury sphygmomanometer Pressures were read to the nearest 2 mm. Concerning the diastolic BP both the point of muffling (phase 4) and the point at which the sound disappeared (phase 5) were recorded in the sitting and lying positions, while in the standing position only phase 4 as recorded. Heart rate was registered in the sitting position.

Laboratory analyses were performed according t the routine methods used at the Central Laboratory Hematocrits were spun in a microcentrifuge. Serum potassium and serum sodium were determined by means of flame photometry Serum chloride was determined titrimetrically Uric acid was determined by an enzymatic method (11)

The participants were informed that they were to receive predominantly alprenolol or chlorthalidone during half the period of the study and predominantly the other agent during the other half of the study in order to find out which suited them best.

Alprenolol (Aptin®) was given 100 mg 4 times daily except during the first two weeks, when half this dosage was given. Chlorthalidone (Hygroton®) was given 50 mg once daily When not given as active substance, alprenolol and chlorthalidone were replaced by placebo (dummies with the appearance and taste of Aptin® and Hygroton® respectively) In addition, 0.75 g potassium chloride in a slow-release preparation (Kalium Duretter®) twice daily was given throughout the study thus during the placebo periods as well. Altogether the participants had to take 7 tablets day during the whole period of the study After each treatment period the subjects returned the bottles, and the remaining tablets ere counted The tablets were supplied by AB Hässle

Follow-up study

Twenty-five omen continued to tak alprenolol after completing the cross-over study Sixteen had alprenolol in the same dosage as during the cross-over study (400 mg/d.), while in nine women ith a less pronounced response to alprenolol the dosage was doubled (800 mg/d). No other tablets were given during the follow-up study BP was measured by the same technique as during the cross-over study

Statistical methods

Conventional statistical methods were used for calculation of mean values and S.E. The significance of difference between sample means was estimated with Student's *t*-test for the means of differences between paired observations. In this way each subject acted as her own control throughout the study Only those subjects for whom observations were obtainable at corresponding points during the study ere included. The differences were considered statistically significant for $p < 0.05$ The calculations were made in an IBM 360/65 computer Mrs Gullbritt Palm has been consultative statistician and responsible for the computer analysis.

RESULTS

Effects on arterial blood pressure

Cross-over study The mean values for systolic and diastolic BP at the different determinations are given in Fig. 2. The randomized group starting with chlorthalidone had a lower initial mean value after the first placebo period, and the difference between the groups increased during the first treatment period. During the second treatment period, when they had changed to alprenolol, both systolic and diastolic BPs were higher than

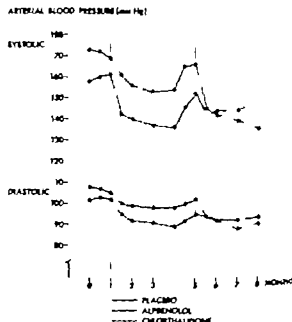


Fig 2. Means of systolic and diastolic BP in the sitting position t the control examinations.

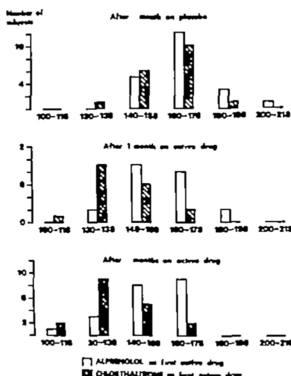


Fig. 3. Systolic BP in the sitting position after the first placebo period and after the first treatment period with alprenolol (21 subjects) and chlorthalidone (18 subjects).

in the other group, who had then changed to chlorthalidone. It will also be seen that most of the reduction occurred during the first month of treatment as regards both alprenolol and chlorthalidone. During the second placebo period there was an increase of the BP but not to pre-treatment values. Thus a hypotensive drug effect seemed to remain at the end of the second placebo period. Only the difference between the first placebo period and the first treatment period, therefore, was used for a comparison of the effect on the BP of alprenolol and chlorthalidone with placebo. The distribution of systolic BP after the first placebo period and after the first periods of alprenolol and chlorthalidone treatment is shown in Fig. 3.

Table I gives group means and means of intra-individual differences of BP in the sitting, lying and standing positions at the end of the first placebo period and at the end of the first alprenolol and chlorthalidone periods, respectively. Both alprenolol and chlorthalidone reduced the systolic and diastolic BPs significantly in all positions as compared to placebo.

Table II shows the difference between alprenolol and chlorthalidone after 3 months of treatment, irrespective of whether treatment commenced with

Table I. *BP in sitting, lying and standing positions during the first placebo periods and during the first treatment periods with alprenolol and chlorthalidone*

P = placebo, A = alprenolol, C = chlorthalidone

Position	Drug		Mean	S. E.	Drug		Mean	S. E.		Difference	S. E.	Probability
Systolic												
Sitting	P	22	169	2.8	A	21	134	3.8	21	15.7	3.0	0.001
Sitting	P	18	161	2.9	C	18	136	3.1	18	25.6	2.2	<0.001
Lying	P	22	170	2.6	A	21	157	3.6	21	13.1	3.0	<0.001
Lying	P	18	161	3.7	C	18	140	3.2	18	20.8	2.5	0.001
Standing	P	22	160	2.8	A	21	152	3.6	21	8.8	3.2	<0.02
Standing	P	18	156	4.1	C	18	134	2.7	18	22.0	4.4	<0.001
Diastolic (phase 4)												
Sitting	P	22	105	1.9	A	21	98	2.4	21	7.5	1.3	<0.001
Sitting	P	18	102	1.6	C	18	89	1.8	18	12.7	1.4	<0.001
Lying	P	22	103	1.7	A	21	96	2.4	21	6.3	1.3	<0.001
Lying	P	18	101	1.6	C	18	89	2.2	18	11.8	1.4	<0.001
Standing	P	22	107	1.6	A	21	100	2.5	21	7.4	2.0	<0.005
Standing	P	18	106	1.6	C	18	92	2.0	18	13.2	1.7	<0.001
Diastolic (phase 5)												
Sitting	P	22	103	2.0	A	21	96	2.4	21	7.3	1.4	<0.001
Sitting	P	18	99	1.6	C	18	87	1.9	18	12.4	1.5	0.001
Lying	P	22	100	1.7	A	21	94	2.5	21	5.7	1.4	<0.001
Lying	P	18	98	1.7	C	18	87	2.2	18	11.2	1.4	<0.001

Table II *BP in sitting lying and standing positions after 3 months' treatment with alprenolol and with chlorthalidone*

Position	Alprenolol			Chlorthalidone			Difference			Probability
		Mean	S. E.		Mean	S. E.		Mean	S. E.	
Systolic										
Sitting	39	151	3.0	40	136	2.3	39	15.9	2.6	<0 001
Lying	39	152	3.1	40	140	2.2	39	11.5	2.6	<0.001
Standing	39	149	2.8	40	134	1 9	39	15.2	3.0	<0.001
Diastolic (phase 4)										
Sitting	39	95	1.6	40	90	1 4	39	5.7	1 4	<0.001
Lying	39	95	1.6	40	90	1.5	39	4.7	1.2	<0.001
Standing	39	99	1.6	40	93	1.3	39	6.5	1 4	<0.001

alprenolol or chlorthalidone. Chlorthalidone reduced the BP significantly more than alprenolol and to about the same degree in all positions after 3 months of treatment. Phase 5 of the diastolic BP was omitted in the Table, as it closely followed phase 4 as shown in Table I.

Follow-up study As can be seen from Table III, BP was essentially the same during a 3 months' follow-up period as during the cross-over study in those 16 women who had unchanged dosage of alprenolol. In those who had the alprenolol dosage doubled there was a further reduction of 14 mmHg for the systolic and 6 mmHg for the diastolic BP The reduction was statistically significant for both ($p < 0\ 05$)

Effects on heart rate

Pre-treatment values of heart rate were almost restored during the post-treatment placebo period, as shown in Fig. 4 The values of both placebo periods can therefore be compared with those of the subsequent periods on alprenolol and chlorthalidone. As seen in Table IV the heart rate was the same during the periods on chlorthalidone and the preceding placebo periods, while alprenolol reduced the heart rate by 10 beats/min as compared to placebo, which is statistically significant ($p < 0\ 001$). The mean intraindividual difference between alprenolol and chlorthalidone was also 10 beats/min. No correlation was found between the effect of alprenolol on the heart rate and its effect on the BP

Effects on weight

As regards heart rate, both placebo periods were considered when comparison was made with the subsequent treatment periods. Alprenolol caused a mean weight increase of 1 0 kg, while weight decreased 1 4 kg during treatment with chlorthalidone compared to the preceding placebo periods (Table IV). Both differences were statistically significant ($p < 0\ 001$). The intraindividual difference between alprenolol and chlorthalidone was 1.8 kg (lower with chlorthalidone, $p < 0\ 001$)

Table III *BP during a follow-up study with alprenolol in unchanged and doubled dosage*

Double-blind study			Follow-up study					
Dosage (mg/d.)		Mean	Dosage (mg/d.)		Mean		Difference	Probability
Systolic								
400	9	165	800	9	151	9	13.6	<0.05
400	16	138	400	16	137	16	1 4	—
Diastolic (phase 4)								
400	9	97	800	9	91	9	6.0	<0.05
400	16	90	400	16	88	16	2.0	—

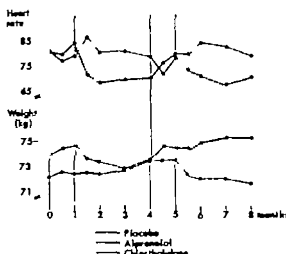


Fig. 4. Heart rate and weight at the control examinations (means).

Effects on laboratory data

Hematocrit did not change significantly as can be seen from Table V. Serum potassium was the same with placebo and alprenolol, but decreased significantly during treatment with chlorthalidone as compared to placebo and alprenolol ($p < 0.001$). Serum chloride behaved in the same way while serum sodium remained essentially unchanged. Serum uric acid increased significantly with both alprenolol and chlorthalidone ($p < 0.001$) and the increase was significantly greater with chlorthalidone than with alprenolol ($p < 0.025$). This difference is still more noticeable when the individual values for uric acid are studied. Thus there were three women with uric acid in serum ≥ 6 mg/100 ml during alprenolol treatment as compared to 11 during chlorthalidone treatment, six of whom had uric acid ≥ 7 mg/100 ml.

Other laboratory data, such as bilirubin in serum, SGOT and leucocytes, did not change during the treatment periods. Two women with mild proteinuria before the study continued to lose the same amount of protein in the urine during the treatment periods. One of these also had a mild diabetes mellitus. Otherwise neither proteinuria nor glucosuria was found.

Control of tablet intake

After each treatment period the remaining tablets were counted. It was found that the percentage of tablets taken decreased with increasing number of daily administrations. Thus chlorthalidone (administered once daily) was taken to an extent of 97% potassium chloride (administered twice daily) to 93% and alprenolol (administered four times daily) to 85%.

Side-effects

One woman discontinued the medication during the alprenolol period. She had a disabling rhinitis, probably an effect of alprenolol.

Seven participants had to reduce the dosage during the first weeks. They complained of headache, dizziness, palpitations of the heart, fatigue or weakness. Five of them were on chlorthalidone, one on alprenolol, and one on placebo. They were all free from symptoms when taking half the dosage, and after some weeks all but one tolerated the full dosage without symptoms.

One woman complained of slight shortness of breath when treated with alprenolol, but treatment could be continued.

DISCUSSION

Previous findings of a significant effect of alprenolol on high BP (4, 19) have been verified in

Table IV. *Heart rate (per min) and weight (kg) during treatment with alprenolol and chlorthalidone and the preceding placebo periods, and mean intraindividual differences*

P = placebo, A = alprenolol, C = chlorthalidone

	Drug		Mean	S. E.	Drug		Mean	S. E.		Difference	S. E.	Probability
Heart rate	P	39	81	2.5	A	39	70	1.9	38	10.4	2.1	0.001
Heart rate	P	37	80	1.7	C	40	80	2.1	37	1.1	1.5	—
Heart rate	A	39	70	1.9	C	40	80	2.1	39	10.3	1.3	<0.001
Weight	P	39	72.1	2.7	A	38	73.4	2.8	37	1.0	0.26	<0.001
Weight	P	39	72.5	2.8	C	39	71.4	2.7	38	1.4	0.26	<0.001
Weight	A	38	73.4	2.8	C	39	71.4	2.7	37	1.8	0.23	<0.001

Table V. *Hematocrit, serum electrolytes (mEq/l) and serum uric acid (mg/100 ml) during treatment with alprenolol and chlorthalidone and the preceding placebo periods, and mean intraindividual differences*

P = placebo, A = alprenolol, C = chlorthalidone

	Drug		Mean	S. E.	Drug		Mean	S. E.		Difference	S.E.	Probability
Hematocrit	P	40	42.2	0 6	A	40	42.4	0.5	40	0.3	0.5	—
	P	39	42.8	0.5	C	40	42.9	0 6	39	0.3	0.4	—
	A	40	42.4	0.5	C	40	42.9	0 6	40	0.6	0.4	—
Sodium	P	40	142	0 4	A	40	143	0.6	40	1.2	0.7	—
	P	39	142	0 4	C	40	142	0.5	39	0 1	0.6	—
	A	40	143	0.6	C	40	142	0 5	40	1 7	0.6	<0.01
Potassium	P	40	4 2	0 06	A	40	4.3	0.05	40	0.1	0.06	—
	P	39	4 1	0.04	C	40	3.4	0 07	39	0 7	0.07	<0.001
	A	40	4.3	0 05	C	40	3.4	0.07	40	0.9	0.08	<0.001
Chloride	P	40	107	0.4	A	40	107	0.4	40	0.3	0.5	—
	P	39	107	0.4	C	40	102	0.6	39	5 1	0.6	<0 001
	A	40	107	0.4	C	40	102	0.6	40	4.8	0.6	<0 001
Uric acid	P	40	3.6	0.2	A	40	4 4	0.2	40	1 0	0 2	<0 001
	P	36	3.8	0.2	C	40	5.0	0.2	36	1 4	0.3	<0 001
	A	40	4.4	0.2	C	40	5 0	0.2	40	0.7	0.3	<0.025

this study. In the dosage given, chlorthalidone reduced the BP significantly more than alprenolol. Chlorthalidone was given in the dose usually recommended in Sweden for the treatment of hypertension. Alprenolol was given in the standard dose for the treatment of angina pectoris. The hypotensive effect of alprenolol as compared to placebo was somewhat higher than for propranolol when propranolol was given in a dosage of 120 mg a day (15) and somewhat lower than for [illegible]lol 120–3 0 mg a day (22).

The necessity of having a fixed dose in a study like this presents difficulties when interpreting the results. It is known that the BP reducing effect of propranolol is dose-dependent (13). A follow-up study on alprenolol in this material showed that an increase of the alprenolol dosage had a further effect on the BP. This means that a BP-reducing effect more similar to that of chlorthalidone might have been obtained if higher doses of alprenolol had been used in this study.

During the cross-over study alprenolol reduced the BP mainly during the first month, but some further decrease was also found during the subsequent months. This is in agreement with what was found for propranolol (12).

During the second placebo period BP did not return to the pre-treatment values of the first period. This was most evident in those who had been treated with chlorthalidone. This may to some degree be explained by the fact that the participants became more accustomed to the circumstances of the clinical visits, but probably a placebo period of one month was too short to eliminate the hypotensive effect of the drugs given earlier. This is in agreement with earlier observations for chlorthalidone (6).

Heart rate was reduced with alprenolol. The effect was about the same [illegible] that found previously with propranolol (15). Prichard et al. (14) found a maximal bradycardic effect with propranolol within [illegible]4 hours. In this study with alprenolol most of the heart rate reducing effect of the drug was recorded at the first clinical examination. The reduction of the heart rate did not cause symptoms and was no therapeutic problem. Chlorthalidone did not influence the heart rate.

Alprenolol caused a moderate but significant weight gain. Similar observations have been made during propranolol treatment (15). Chlorthalidone reduced the weight significantly.

Hypokalemia is a well-known problem when using saluretics. The hypokalemic effect of chlorthalidone has been found to be about the same as for other saluretics (7, 10, 21). The daily supply of 1.5 g of potassium chloride in this study did not prevent a marked decrease in the serum potassium concentration. However, no clinical symptoms of hypokalemia were found. Serum potassium rose during the placebo period after chlor

thalidone withdrawal, which was also noted by Healy et al. (6). It was essentially the same during the alprenolol periods as during the placebo periods.

The hyperuricemic effect of saluretics is another problem (7 18), which was obvious in this study as well. Alprenolol also increased serum uric acid significantly but not to the same degree as chlorthalidone. Interference with uric acid excretion from the renal tubules is considered to be the reason for the hyperuricemic effect of the saluretics (18) though there is probably another explanation in the case of alprenolol, possibly the effect on the renal circulation secondarily to its β-blocking effect.

Alprenolol seemed well-suited for the treatment of mild hypertension. In the dosage given in this study its hypotensive effect was less than that of chlorthalidone, but a more marked effect on BP was obtained when the dosage was increased. Subjective symptoms which could be interpreted as side-effects were few and as a rule transient with both alprenolol and chlorthalidone. In comparison with chlorthalidone, alprenolol had no effect on serum potassium and a more moderate effect on serum uric acid.

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THE EFFECT OF DIGITALIS ON THE HEART RATE DURING EXERCISE IN PATIENTS WITH ATRIAL FIBRILLATION

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Abstract. Twenty-eight clinically compensated cardiac patients with atrial fibrillation have been investigated with the graded exercise test. T enty-four patients had one test with lower dose of digitalis and one with a higher dose, both dose rates being within the therapeutic range. Four patients performed one exercise test with and one without digitalis. The heart (ventricular) rate attained at a given exercise load was significantly lower (with a difference of 5–25 beats/min at the different loads) with the higher dose. This was interpreted as beneficial effect on working capacity. The individual beat-to-beat variability of heart rate was not influenced. The beneficial effect of an increase in digitalis dose thus seems to depend on decrease in average ventricular rate during exercise and not on decreased degree of arrhythmia.

In a cardiac patient with atrial fibrillation one main therapeutic effect of digitalization is slowing of the ventricular rate. The mode of action is still discussed and not completely understood (7). A dual mechanism has been suggested, one working through the vagal system and the other being of non-vagal nature (3).

During physical exercise in a normal individual the heart rate increases. At first this is due to a decreased vagal tone and then also to increased sympathetic tone. During gradually intensified physical exercise patients with atrial fibrillation increase their average ventricular rates. Sometimes this increase is relatively regular and more or less linear in relationship to the exercise loads, indicating a well controlled change in atrio-ventricular transmission. Sometimes, however, the rise in heart rate occurs in an irregular and non-linear fashion. According to clinical experience this reaction appears to be influenced by the degree of digitalization (1, 9).

The purpose of the present paper is to analyse the effect of different digitalization levels on the heart rate reaction to a standardized exercise test in patients with atrial fibrillation.

MATERIAL

The composition of the case material is shown in Table I. The total number of patients was 28, 15 males and 13 females. There was no selection except that the patients belonged to hospital population and that there as clinical reason to perform an exercise test. As rule the test was made in an attempt to evaluate the patient's working capacity before consideration for valvular surgery.

In Table II the material has been divided into three groups A, A_1 and B. Group A, comprising 24 patients, was tested on two dose levels of digitalis drug. Subgroup A_1 represents the 14 cases from group A, who at both tests had one and the same drug. Group B, four patients, had no digitalis at the first test but only at the second.

METHODS

The digitalis drugs administered were mostly digoxin (Lanacrist®) and digitoxin (Digitrin®), but other preparations were also given. The technique used with regard to the orthostatic and the exercise tests has been described previously (1). The first exercise test in patient was continued until the appearance of abnormal symptoms and signs according to the judgement of the responsible physician. The second test was usually interrupted at the same load levels as in the first test and, accordingly does not necessarily reflect near-maximal level as the first test usually does. Two tests were performed on each patient, the first as rule with maintenance dose ("lower dose") and the second with an increased amount of digitalis ("higher dose") (4). In few cases the order of the change was the opposite. There was no incidence of digitalis intoxication as judged by the clinical picture.

A preliminary presentation of this report was given at the meeting of Svensk Internmedicinsk Förening in Uppsala, Sweden, Sept. 1970.

Table I. *Composition of the case material*

Diagnosis	No. of pats. Male	Female	Total	Age (y.) Mean	Range	Heart volume (ml/m² BSA) Mean	Range
Mitral stenosis	4	5	9	46	37–53	654	540–800
Mitral insufficiency	1		1		53		650
Mitral stenosis + insufficiency	3	6	9	47	36–52	676	530–865
Aortic stenosis + insufficiency	1		1		49		810
Mitral + Aortic valve disease	4	2	6	41	32–48	735	580–1000
Three valves disease	2		2	45	38–52	1135	1040–1230
Entire material	15	13	28	45	32–53	718	530–1230

The time between the two tests was usually short but varied from a few days, in 8 cases, up to 20 months in one case. No essential change with regard to clinical status, functional capacity judged by clinical methods, heart volume or corrective surgical procedures had occurred in any of the patients between the two tests.

The individual-average ventricular rate was measured from at least 20 R-R intervals, in most cases 30 intervals. The statistical calculations were carried out on intervals and not on rates according to standard methods, assuming a normal probability distribution of the intervals. In certain cases this may introduce an error as intervals may have a bimodal distribution (10). The response to a work test was expressed as the load giving a ventricular rate of 110 min, W_{130}, and as the highest load performed for 6 min, W_{max}. The mean values of the heart rates at 2, 4 and 6 min on the appropriate load were used for the calculations of W_{130}. In a few cases W_{130} had to be obtained by interpolation (assuming a linear relationship between work load and heart rate within the interval).

RESULTS

The results were in principle the same in groups A, A and B.

At rest. In all cases except two, the ventricular rate was slightly lower with the higher digitalis dose than with the lower dose. The group-average change in resting heart rate was small, however and did not exceed 2 beats/min in the different groups. The difference is not statistically significant.

Orthostatic tests. The group-average ventricular rate was moderately lower with the higher digitalis dose, the difference amounting to 8 beats/min in group A and 5 in group B.

Exercise tests. The individual-average ventricular rate was considerably lower with the higher digitalis dose in the majority of cases at all load levels. The mean decreases in heart rate are presented in Table III and varied between 5 and 15. The results are also expressed as changes in R-R intervals (Table III). For the sum of all tests the difference was statistically highly significant ($p < 0.001$).

These findings clearly show an increased physi-

Table II. *Result of different dosage of digitalis*

No. of pats.	Group	Digitalis regimen	Resting heart rate Mean	Range	Orthostatic test Mean	Range	W_{130} Mean	Range	W_{max} Mean	Range	Final heart rate Mean	Range
14	A	Higher dose	73	48–86	83[a]	54–104	190[b]	20–480	307	150–600	146	88–195
		Lower dose	74	46–90	91	58–135	148[b]	10–350	304	100–450	152	114–195
4	A	Higher dose	76	48–130	84	54–140	179[d]	20–480	296	50–600	147	88–195
		Lower dose	78	46–110	92	58–135	139[d]	10–450	283	50–500	157	104–199
4	B	Digitalis	78	63–96	88	78–96	213	50–350	375	250–550	141	107–169
		No digitalis	79	70–90	93	66–114	98	40–200	363	200–550	148	113–164

One patient did not have the test.
[b] W_{130} could not be estimated in one patient.
Two patients did not have the test.
W_{130} could not be estimated in three patients.

Table III. *Mean R R intervals and heart rates on various loads with lower and higher dose rates of digitalis*

L lower dose; H = higher dose. S.D in parentheses

Work load (kpm/min)	No. of tests	R–R interval (csec) L	R–R interval (csec) H	R–R interval (csec) Difference	Heart rate (beats/min) L	Heart rate (beats/min) H	Heart rate (beats/min) Difference
50	1	49.6 (10.5)	54.3 (10.7)	4.7 (13.3)	121	110	11
100	9	49.3 (10.1)	58.1 (12.5)	8.8 (11.3)	122	103	19
150–200	10	48.8 (9.5)	50.9 (11.1)	2.1 (14.1)	123	118	5
250–300	7	38.8 (7.4)	46.3 (9.8)	7.5 (9.2)	155	130	25
> 350	7	36.7 (7.8)	41.8 (9.0)	5.1 (9.4)	163	144	19
> 50	45			5.2 ** (11.6)			

cal work capacity when expressed as load at a given heart rate, e.g. W_{140}, with the higher dose of digitalis.

At the second exercise test the aim was mainly to repeat the original test at the same load level. Consequently there was no significant difference in W_{max} between the two tests.

For each patient the beat-to-beat variability in heart rate was calculated from 20–30 serial R R intervals and expressed as variance. Group-average variances were then calculated for different exercise loads and digitalis doses (Table IV).

For each heart rate the variability in R-R intervals was found to be the same at the higher and lower doses of digitalis (Fig. 1).

Table IV. *The ventricular rate expressed as R R interval, mean and S.D of the mean, with higher (H) and lower (L) dosages of digitalis*

Work load (kpm/min)	No. of tests per load	Mean of R R interval (csec) H	Mean of R R interval (csec) L	S.D. of R–R interval (csec) H	S.D. of R–R interval (csec) L
50	11	53.4	48.6	10.7	10.5
100	8	60.7	49.6	12.5	10.1
150–200	8	51.4	48.3	11.1	9.5
250–300	4	47.7	35.2	9.8	7.4
> 350	6	42.5	36.9	9.0	7.8

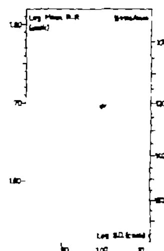


Fig. 1. Relationship between the heart rate, expressed as the mean R-R interval, and the variance of the R-R intervals.

DISCUSSION

In normal subjects and in cardiac patients with sinus rhythm and not in heart failure, digitalis often does not influence the heart rate, either at rest or during exercise (8, 13).

In compensated cardiac patients with atrial fibrillation the effect of the digitalis dosage on the heart rate reaction to exercise is not completely understood (5, 9, 11, 12). Holmgren et al. (5) reported a regular and approximately linear relationship between ventricular rate and different exercise loads as an average for a group of patients with mitral valve disease and atrial fibrillation. Varnauskas et al. (11) in accordance with others (2, 6), on the other hand, found that on

exercise the individual-average ventricular rate may increase much more irregularly during atrial fibrillation than during sinus rhythm. The non-linearity was found to be diminished by digitalis but not abolished. The latter authors stressed that even at a small exercise load an extremely high ventricular rate may often be obtained, which remains almost unchanged at increased loads although with an increasing cardiac output. Their conclusion was that the influence of extracardiac factors on the ventricular rate is governed by another mechanism than normally. In such case the heart rate reaction to submaximal exercise would not give a true picture of the functional capacity.

The present problem may be divided into two parts: does the digitalis dose level influence 1) the linearity of the individual-average relationship between work load and heart rate in patients with atrial fibrillation, and 2) the individual beat-to-beat variability at a given heart rate?

In a previous report (1) we have studied the linearity in a large group of digitalized patients with atrial fibrillation undergoing exercise tests. The increase of heart rate was found to be slightly more pronounced between rest and the starting load in comparison with the following increases at successive loads, but in many cases the change in heart rate was relatively linear. Patients in a serious clinical condition and with a very low working capacity tended to have the irregular type of ventricular rate increase during exercise more commonly than patients in a better clinical condition. On the average our material represented fairly severe heart disease and both types of reactions to exercise loads were found (cf. 5, 11).

The results of the present study demonstrate that the heart rate at rest was not influenced by an increase of the digitalis dose in a group of clinically compensated cardiac patients with atrial fibrillation. A similar observation has recently been made by Redfors (9).

However, the increase of the dose of digitalis caused a significant decrease in heart rate at a given exercise load. This must reasonably mean that the increased digitalis dose improved the working capacity. This is true also in a patient with the non-linear type of heart rate reaction to exercise if it is assumed that—in accordance with the general rule—a low heart rate is more advantageous than a high heart rate, at a given cardiac output, because of a lower myocardial oxygen consumption.

The findings also imply that the increased digitalis dose results in a better linearity between exercise load and heart rate, which means that the diagnostic value of a standard exercise test improves with digitalization.

From the practical point of view another conclusion should be pointed out, too. It is obvious from the present material that fully compensated cardiac patients with atrial fibrillation who are on an ordinary therapeutic dose of digitalis, often may not be optimally digitalized. The optimum level of digitalis dosage may therefore be higher in patients with atrial fibrillation than in comparable patients with sinus rhythm in whom the inotropic digitalis effect is the main objective.

The variance of the individual R R interval at a given ventricular rate was found to be uninfluenced by the digitalis dose. This must mean that digitalis does not have any true antiarrhythmic activity in itself. The regularizing property of digitalis is apparently secondary to its slowing effect on the heart rate.

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CHANGES IN THE LIPOPROTEIN PATTERN DURING TWO YEARS FOLLOWING MYOCARDIAL INFARCTION

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Abstract. In a previous investigation a normal lipoprotein pattern was demonstrated in 51% of 85 men during the first three months after myocardial infarction. Hyper-β-lipoproteinaemia (type II pattern) was found in 22% and hyper pre-β-lipoproteinaemia (type IV) in 27%. In a follow-up study approximately 25 months after the myocardial infarction we found a normal lipoprotein pattern in 43%, hyper-β-lipoproteinaemia in 31% and hyper-pre-β-lipoproteinaemia in 26% of 58 men. During the observation period there has been a significant elevation of the serum cholesterol levels in all groups and of the triglyceride level in type IV patients.

Based on the classification of Fredrickson et al. (4) we have previously made a study of the lipoprotein pattern during the first three months following acute myocardial infarction (3).

In order to study changes, if any, in the serum lipids in untreated patients, the lipoprotein pattern has been controlled approximately two years after the first attack of myocardial infarction.

MATERIAL AND METHODS

Eighty-five men who had their first experience of myocardial infarction below the age of 70 years were included in this study. The usual criteria (11) for the diagnosis of myocardial infarction were applied. Patients with liver, kidney and endocrine diseases apart from diabetes mellitus were excluded.

At discharge from the hospital the patients were not recommended any special diet, apart from weight reduction if necessary.

Our first classification was based on blood samples taken on the 2nd and the 9th day and 3 months after the myocardial infarction.

In this follow-up study serum lipids were examined in the fasting state approximately 2 years (average 25.5 months) after the myocardial infarction. Twenty of the patients had died, six did not turn up, and one was withdrawn because of renal disease. The group was accordingly reduced to 58 patients. Serum cholesterol (Liebermann Burchard reaction a.m.) (13) and triglycerides (7) were determined, and plasma lipoprotein electrophoresis on paper (8) was carried out. The laboratory methods were controlled with known sera. The cholesterol method has been compared with the methods used in 10 Norwegian departments of clinical chemistry. Our results were 6–8% higher than the average values in the 230–325 mg/100 ml range, 11% higher above 500 mg/100 ml.

The patients were classified into four groups according to serum lipid values and the serum lipoprotein electrophoresis pattern: normal, Fredrickson and Lees' type II (with or without pre-β-lipoproteinaemia), type IV and an indefinite group in which it was impossible to define the pattern.

Normal: cholesterol < 325 mg/100 ml and triglycerides < 150 mg/100 ml in all four tests.

Type II (hyper-β-lipoproteinaemia): cholesterol > 350 mg/100 ml in at least one of the tests or 326–350 mg/100 ml in at least two of the tests and at the same time increased β-lipoprotein on electrophoresis, or (with pre-β-lipoproteinaemia) *in addition to increased cholesterol* values, serum triglycerides > 150 mg/100 ml and simultaneously increased β and pre-β-fractions on lipoprotein electrophoresis.

Type IV (hyper-pre-β-lipoproteinaemia): triglycerides > 200 mg/100 ml in at least one of the tests or 150–200 mg/100 ml in at least two of the tests and increased pre-β and normal β-lipoprotein on electrophoresis.

Indefinite: with either cholesterol < 325 in all tests and triglycerides 151–200 mg/100 ml in one of the tests or cholesterol 326–350 mg/100 ml in one of the tests and triglycerides < 150 in all tests, and without definite changes in the lipoprotein pattern on electrophoresis.

RESULTS

The results are summarized in Table I.

Normal. Among the 26 patients originally classified as normals only 17 had unchanged lipoprotein pattern. Of the nine patients with altered pattern four showed type II and one type IV pat-

cholesterol level was the most important risk factor

On the other hand an Australian investigation shows that the serum triglycerides are more important than cholesterol (10)

Accordingly there still seem to exist great differences of opinion on the evaluation of the importance of the different serum lipids. Until more information has been collected, it seems justifiable to establish the lipoprotein pattern in patients with coronary heart disease. The examinations should not be performed during the first weeks after a myocardial infarction, when the lipid levels are lower than afterwards.

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THE HAEMODYNAMIC EFFECT OF INTRAVENOUS INJECTION OF LEVODOPA

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Abstract. Levodopa has been given in dose of 100 to 200 mg into the pulmonary artery of eight patients with heart disease undergoing routine catheterization studies. The cardiac rate, the systemic and pulmonary arterial pressures, the cardiac output, the stroke volume and peripheral resistance ere calculated. Tachycardia was found in all patients. Ventricular premature contractions were seen in tw patients. An initial fall followed by rise in systemic arterial pressures; rise in cardiac output, rise in stroke volume, and fall in peripheral resistance were observed in the majority of patients. Nausea was the commonest side-effect.

Levodopa (L-dopa, L 3 4-dihydroxyphenylalanine) has brought a better hope in the medical management of parkinsonism. The basis of this treatment had arisen from the observations that the post mortal dopamine content of the basal ganglia is greatly reduced in individuals with parkinsonism (8). However dopamine could not be used in the treatment of parkinsonism as it could not pass through the blood-brain barrier (6). Levodopa, precursor of dopamine, was found to do so. A pronounced improvement in the symptomatology of patients with parkinsonism of levodopa was shown by a number of workers (7 12). Dopamine is a sympathicomimetic substance with powerful circulatory effects (11). Circulatory side-effects due to the L-dopa have been observed in patients on oral treatment (7). However the number of studies on the haemodynamic changes due to levodopa on i.v administration is limited (2). This study was made in order to observe the circulatory and haemodynamic changes when the drug is administered i.v in patients with organic heart disease.

MATERIAL

The study comprised eight patients, six females and two males, with organic heart disease who were on routine catheterization. The age of the patients varied between 40 and 77 years, the average being 51 4 years. Six of them belonged to cardiac functional group II and two to functional group III (N Y.H.A.). One patient had atrial septal defect and the remainder chronic rheumatic valvular heart disease.

METHODS

Right heart catheterization was performed with Cournand catheter Left heart catheterization was done by the Seldinger technique through the femoral artery using polythene radio-opaque catheter hich as placed in the aorta for pressure recordings.

Levodopa in physiologic saline solution containing mg/ml was injected over period of 10 min through the Cournand catheter placed in the pulmonary artery The solution was warmed to 40°C before injection so as complete to dissolve the drug. The total amount of the drug given to different patients varied between 100 and 200 mg (average 144).

During the initial 10 min of the examination the aortic pressures were recorded every minute. Thereafter the aortic and the pulmonary arterial pressures were recorded up to 30 min. ECG was recorded throughout the examination to note the cardiac rate and arrhythmias.

The cardiac output was calculated employing Fick principle before injection of the drug, and 12 to 15 and 28 to 31 min after the start of the injection.

The volume of the expired air was measured using Tissot spirometer, and oxygen consumption was calculated from gas analyses by the micro-method of Scholander

RESULTS

In all the patients mild to moderate circulatory changes were observed. The cardiac rate showed a consistent rise from the resting levels. The rise was marked and statistically significant ($p < 0.05$) at 12 to 15 min after the start of the injection (Fig. 1). Premature ventricular beats were observed in two patients.

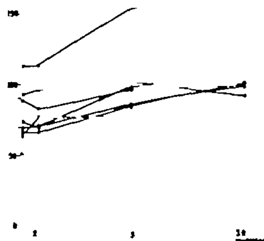


Fig 1 Heart rate in eight patients before, 2, 15 and 30 min after i. injection of levodopa.

Systemic arterial pressure showed a variable change during the first minutes. There was a sudden transient fall in systolic pressure in five patients. After the initial fall the systolic pressure showed a tendency to rise, and in half of the cases the pressure level at 13 min was higher than the resting level. At the end of the examination the pressure showed a tendency to fall (Fig. 2). The

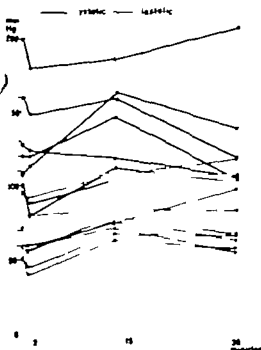


Fig. 2 Systemic arterial pressure in eight patients before, 2, 15 and 30 min after i. injection of levodopa.

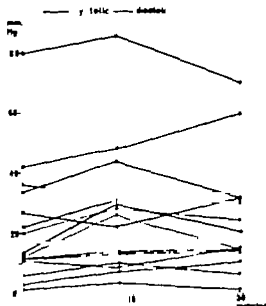


Fig 3 Pulmonary arterial pressure in eight patients before, 15 and 30 min after i.v. injection of levodopa.

diastolic pressure in the aorta showed a sudden fall in 7 of 8 patients during the first 5 min, followed by a tendency to rise in all patients.

The pulmonary arterial pressure also showed small and variable changes. A tendency to rise was seen in 5 patients at 13 min, later showing a tendency to fall (Fig. 3).

The cardiac output showed a slight but consistent increase in all the patients at 13 min (Fig. 4). The stroke volume also showed a moderate

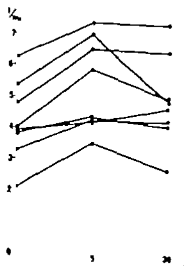


Fig 4 Cardiac output in eight patients before, 15 and 30 min after i. injection of levodopa.

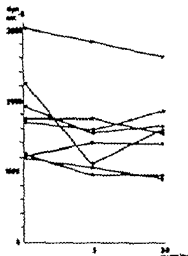


Fig. 5. Systemic arterial resistance in eight patients before, 15 and 30 min after i.v. injection of levodopa.

rise in seven patients at 15 min with a tendency to fall later. The systemic arterial resistance decreased in 6 of 8 patients (Fig. 5).

The most common side-effect was nausea, which was experienced by five patients, in two accompanied by vomiting. The nausea was severe at 10 to 15 min after the start of the L-dopa infusion. Serious arrhythmias were not seen. Two patients had ventricular premature contractions.

DISCUSSION

Cardiovascular side-effects have been reported in patients with parkinsonism on oral treatment with L-dopa. The most frequent side-effects have been hypotension (4, 5), cardiac arrhythmias (7, 12) and occasionally hypertension (5).

We have tested the cardiovascular effect of a moderate i.v. dose of L-dopa in patients with organic heart disease undergoing routine cardiac catheterization. The i.v. route of administration was chosen because the patients stayed for a limited period only in the hospital. As nausea and vomiting were frequent side-effects, and the tendency to nausea seems to be dose related (7), we found it unethical to test the effect of larger doses.

The effects on the cardiovascular system observed were slight. No serious complications were seen. Parks et al. (11) observed in dogs atrial as well as ventricular tachyarrhythmias after i.v. administration, probably due to accumulation of catecholamines. In patients treated with L-dopa paroxysmal supra-ventricular arrhythmias and premature ventricular contractions have been reported (7, 12). In the present investigation only two patients had ventricular premature beats. But tachycardia was observed in all.

The BP showed a tendency to fall in some patients during the initial 5 min after injection and to rise later to values higher than the control values (pre-examination resting values). The initial fall of BP in our patients could be due to the same mechanisms as the dopamine effect. A slight increase in cardiac output and stroke volume probably indicates the increase in the levels of catecholamines due to L-dopa, which is a precursor (9) to dopamine which has been considered to possess a positive inotropic effect (1, 10). Our observations seem to indicate that treatment with L-dopa is not particularly dangerous in patients with organic heart disease. It must, however, be stressed that only a limited number of patients have been examined, the dose of L-dopa has been moderate, and only the immediate effects after i.v. administration have been observed.

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EXTRACORPOREAL IRRADIATION OF THE BLOOD

Effect of Varying Transit Dose on the Degree and the Rate of Development of Lymphopenia

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Abstract. During the last three years a total of 80 patients have received extracorporeal irradiation of the blood (ECIB) prior to kidney transplantation. The irradiators were developed and fabricated by the Danish Atomic Energy Commission, Risø. The main data from three types of equipment—stationary and mobile gamma units and portable beta units—are presented. For the total material the average duration of ECIB was 103 h, the mean transit dose 295 rads and the mean total dose 42 850 rads. The lymphocyte concentration was reduced from 1 350 ± 526 before to 335 ± 122 after ECIB. According to the transit dose the patients were divided into three groups. Group I (20 patients) received a mean transit dose of 96 rads, and a mean total dose of 21 690 rads. Group II (21 patients) received a mean transit dose of 359 rads and an average total dose of 50 100 rads. Group III (20 patients) received an average transit dose of 432 rads and an average total dose of 54 420 rads. The rate of development of lymphopenia was significantly slower in group I than in groups II and III, but reduction of the lymphocyte concentration to the same degree (about 30 % of pretreatment value) was observed in all three groups. At 15 000 rads the percentual reduction was significantly larger in group I than in groups II and III. There was no correlation between flow rate, calculated as the number of patients' blood volumes radiated per hour, and the rate of development of lymphopenia. After cessation of ECIB the lymphocyte concentration was followed in those patients who were not transplanted immediately. In 9 patients belonging to groups II and III the concentration remained constant at 30 % of pretreatment value during the first 8 months. At 12 months it had increased to 50 %. In 6 patients, belonging to group I, the lymphocyte concentration increased to 50 % three months after cessation of ECIB. The practical conclusion of the present study is that prolonged lymphocyte reduction is obtained with ECIB, with high transit doses resulting in high total radiation doses which caused hemolysis. If the total radiation doses are reduced by reduction of transit doses (100 rads), lymphopenia is obtained without hemolysis, but ECIB has to be repeated at more frequent intervals in order to maintain lymphopenia.

The lymphocytes are the most radiosensitive cells in the peripheral blood (13); therefore extracorporeal irradiation of the blood (ECIB) has been proposed by Cronkite et al. (3), and used by different groups (10, 17), as a method for immunosuppressive therapy before transplantation. In a previous paper (17) it was reported that a pronounced and persistent lymphopenia developed in all patients who were treated with ECIB before kidney transplantation, with a mean transit dose of 435 rads and an average total radiation dose of 54 600 rads. However, this schedule caused damage to the red blood cells, resulting in increased requirement for blood transfusions. If the total radiation dose is reduced below 30 000 rads, no measurable hemolysis is caused by the treatment (11, 12). The total radiation dose is calculated as the product of the transit dose and the number of blood volumes passing the radiation field, therefore, keeping the number of radiated blood volumes constant, the radiation dose can be reduced if the transit dose is reduced. The killing dose for the lymphocytes in humans has not been clearly defined. It is therefore the purpose of the present paper to examine the effect of low transit doses on the degree and the rate of development of lymphopenia in humans.

MATERIAL AND METHODS

In the period between Febr. 1, 1968 and March 1, 1971 a total of 80 patients, 42 men and 38 women, received ECIB as immunosuppressive therapy prior to kidney transplantation. The age of the patients varied from 12 to 59 years. All patients were uremic; 76 were on chronic intermittent

Table I. *Main irradiator specifications*

	ECIB equipment		
	I stationary	II mobile	III portable
Radiation source	Co–60	Cs–137	Sr–90/Y–90
Strength (Ci)	840	4 000	3
Energy levels (MeV)	1.17 & 1.33γ	0.66γ	max. 2.27β
Half-life (y.)	5.26	29.4	28
Irradiation channel (no.)	2–4	2	1
Blood tube			
Material	Silastic		
Diameter (mm)	Outer 5.0, inner 3.0		
Transit dose			
Variation	Fixed levels	Continuous	Stepwise
Range[a] (rads)	10, 30, 150, 300	0–800	10, 33, 56, 79, 85
Unit weight (kg)	2 400	450	3.5
Max. *external radiation levels*			
At surfaces (mR/h)	2	2	000
At 1 m distance (mR/h)	0.75	0.75	3

[a] Related to 100 ml/min blood flow.

dialyses treatment, 4 were treated with dietetic restrictions only.

The technique of ECIB has been described elsewhere (1). In brief, the blood from the patient's arterio-venous shunt (or fistula) is led through an external blood tube (silicone rubber, 5 mm/3 mm) passing the radiation field of the therapy unit. In most cases a blood pump (Travenol) was used to obtain a constant blood flow which was checked by measuring the time for passage of a micro-bubble of air over a known length of blood tube. During ECIB the patients were heparinized, initially with 5 000 I.U. followed by 2 000 I.U. approximately every hour guided by the clotting time, measured in glass, which was maintained for more than 60 min. Usually ECIB was given 6–10 hours per day 3–4 times per week, sometimes in connection with hemodialysis treatment. The duration of one series of ECIB was about 3 weeks. Eight of the 80 patients were given ECIB only during hemodialysis twice weekly and in these cases the treatment period was somewhat extended.

Of the 80 patients 78 were treated with ECIB in gamma units and two in a new portable beta unit. The irradiators were developed and manufactured by the Danish Atomic Energy Commission (DAEC) Research Establishment Risø (18). The main data from the three types of equipment are summarized in Table I.

A *stationary gamma unit ECIB I* was developed in 1966 for research purposes, primarily for treatment of lymphatic leukemia (1). The unit had major drawbacks. First, the feeding of blood tubes into the irradiation channels was a rather elaborate procedure. Secondly the apparatus could not be used in connection with hemodialyses, a fact that caused prolongation of the total period of extracorporeal circulation. Finally it was necessary to break the isolation of the newly transplanted patients for ECIB treatment in the immediate postoperative period.

Thirty-six of the 80 patients were treated with ECIB I. The unit was withdrawn from operation in 1970.

A *mobile gamma unit ECIB II* was developed in 1969 (Figs. 1 and 2). This unit can be used in connection with hemodialyses and can be transported to the isolation room of newly transplanted patients. The blood tube is mounted on a flexible insert which can be introduced at a variable length in one of the irradiation channels. In this simple way the transit dose, determined by the position of the insert in the channel, can be varied over the entire range from zero to approximately 800 rads at a blood flow rate of 100 ml/min. Due to the integrated shielding the external surface radiation level is kept below 2 mR/h.

Forty-two of the 80 patients have been treated with ECIB II.

Although ECIB II thus has certain advantages over ECIB I, its use still required that the patients were lying in bed with their shunt (or fistula) extremities fixed in a rather stationary position. In practice it was therefore not possible to extend the individual session of treatment over more than 12 hours.

A *portable beta unit ECIB III* (Fig. 3) was developed in Feb. 1971 with the main aim of permitting prolonged therapy sessions without unacceptable physical discomfort to the patients. The ultimate goal was to continue treatment for a sufficiently long period to obtain acceptable lymphopenia with a single therapy session. The blood tube can be mounted in one to five windings on an interchangeable support structure in the irradiation channel between the sources (Fig. 4). The transit dose can be varied stepwise (10, 33, 56, 79 and 85 rads) merely by changing the number of windings of the blood tube passing the channel. The external surface radiation level is 2 000 mR/h, but because the irradiator is removed from the patient's body when in bed or sitting in a chair the total dose to the patients is below 700 mR. The staff and visitors are in-



Fig. 1 Mobile gamma unit ECIB II.



Fig. 3 Portable beta unit ECIB III.

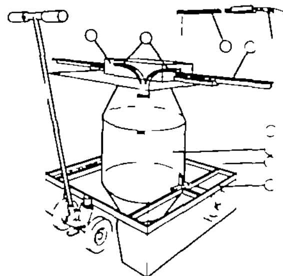


Fig. 2 Mobile gamma unit ECIB II schematically. *1*) T independently operated irradiation channels. *2*) Flexible insert ith 5 mm/3 mm plastic blood tube. *3*) Support guide for flexible insert. *4*) Extracorporeal shunt. *5*) Top shield. *6*) 4 000 Curie Cs-137 source. *7*) Steel-encased main lead shield. *8*) Sheet metal cabinet. *9*) Base plate with heels.

structed to keep at distance of one metre from the unit, and so far except for the patients, nobody has been exposed to an overdose.

Preliminary data on tw patients ho were radiated continuously for about 160 hours about use of blood pump or heparin re presented.

ECIB III is still an intermediate prototype. The final design, especially with respect to shielding, awaits more detailed information about the least transit dose which ill permit development of desirable lymphopenia ithin reasonable time.

Determination of transit dose as done by thermoluminescence dosimetry (18).

Leucocytes and differential counts were performed daily.

The statistical calculations were made by NEUCC Lundtofte by means of the least squares method.

Definitions

( ) Transit dose is defined as the radiation dose received by any item hile in transit through the radiation field. Transit dose = *R* (*V* *F*) (rads). *R* = the dose rate of the source. *V* = the volume of blood in the coil within the radiation field (ml). *F* = the blood flow rate (ml min).

(*b*) The number of blood volumes radiated is calculated from the measured blood flow rate through the irradiator and an assumed blood olume of 7% of total b. wt. The number of blood volumes radiated = (*F* *T*) (BV 1 000), here *F* = blood flow rate, *T* the duration of ECIB (min), *BV* = the blood volume (l).

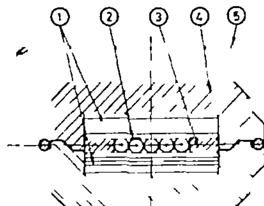


Fig. 4. Portable beta unit ECIB III, on cross section. *1)* 2 1.5 Curie Sr-90/Y-90 sources with primary shielding. *2)* 5 mm/3 mm elastic blood tube. *3)* Tube support. *4)* Secondary lead shield. *5)* Casing.

(*c*) Total radiation dose is calculated as a product of transit dose and number of blood volumes radiated. Total radiation dose = *TD* × no. blood volumes radiated (rads).

RESULTS

Development of lymphopenia in the total material. For the 78 patients treated in the gamma units the mean duration of one series of ECIB was 103 h (43–174), the average transit dose was 295 rads (50–650) and the average number of blood volumes passing the radiation field was 170 (32–188). The mean total radiation dose was 4 850 rads (9 900–103 650). The lymphocyte concentration per μl blood was 1 350 ± 526 (1 S.D.) before and 335 ± 122 (1 S.D.) after ECIB.

The decrease in the lymphocyte concentration, in per cent of pretreatment values, during ECIB is presented in Fig. 5. After 30 000 rads in total radiation dose the lymphocyte concentration is reduced to 30% of the value before ECIB. During the continuous irradiation only a slight further decrease in the lymphocyte concentration was observed within the total dose limits presented in Fig. 5.

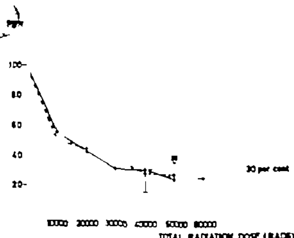


Fig. 5. Lymphocyte concentration in the peripheral blood during ECIB. Ordinate: lymphocyte concentration in per cent of pretreatment value. Mean values ± S.D. Abscissa: total radiation dose (rads). The dashed line represents the mean value in 14 patients who received total radiation dose of 60 000 rads.

Development of lymphopenia in relation to varying transit doses. Sixty-one of the 78 patients treated in the gamma units are divided into three groups according to transit dose (Table II). Group I (20 patients) received a mean transit dose of 96 ± 18 rads, group II (21 patients) a transit dose of 359 ± 19 rads, and group III (20 patients) a transit dose of 432 ± 40 rads. (Seventeen of the 78 patients are excluded. In two the data were insufficient. Five patients received a mean transit dose of 235 rads and two a transit dose of 650 rads. In both groups the observations were too few to permit statistical calculations. Eight patients were treated only during hemodialysis 10 hours, twice weekly. The transit doses employed were: 50 55 100 100 100 320 380 and 500 rads. For all these patients there was a tendency to a slower decrease in the lymphocyte concentration during ECIB compared with patients treated with the same transit doses, but with briefer intervals.)

The decrease in the lymphocyte concentration in per cent of pretreatment value, as a function of total radiation dose in the three groups is presented in Fig. 6. At 15 000 rads the mean lymphocyte level was significantly lower in group I than in group II ($t = 1.75$, $p < 0.1$) or group III ($t = 2.78$, $p < 0.01$).

In Fig. 7 the decrease in the lymphocyte concentration in the three groups is illustrated as a function of the number of blood volumes radiated. By means of the least squares method the curves can be dissolved into two parts, a descending and a horizontal part. The slope of the descending part of the curves is significantly steeper in groups II and III than in group I ($t = 3.17$, $p = 0.004$). Provided that the reduction in the lymphocyte concentration in the blood reflects the behaviour of

Table II. *Data on the three groups of patients who received different transit doses*

Group ..	I	II	III
No. of patients	20	21	20
A transit dose (rads)	96±18	359±19	432±40
A no. of blood volumes radiated	203±58	139±43	130±35
A total radiation dose (rads)	21 680±6 650	50 100±13 690	54 420±12 156
A duration (hours)	91±37	106±25	106±30
A lymphocyte concentration per μl blood			
Before ECIB	1 290±520	1 430±540	1 275±300
After ECIB	360±123	315±103	295±85

the total lymphocyte pool of the organism, it appears that about 70% of the lymphocyte population can be removed by ECIB Furthermore it appears that the concentration of these lymphocytes is halved after 43 radiated blood volumes in group I and about 20 radiated blood volumes in groups II and III. About 30% of the lymphocyte pool is apparently not removed in spite of continuous ECIB within the limits of the present study This "base level" was the same in all three groups.

Development of lymphopenia in relation to number of blood volumes radiated per hour In order to examine the effect of blood flow rate on the rate of development of lymphopenia it was necessary to study variations in blood flow rate at fixed transit doses. Therefore, the patients were divided into two groups. Group A (19 patients) received a mean transit dose of 99 rads (85–120) and the number of blood volumes passing the radiation field per hour varied from 1.2 to 4.6. Group B (38 patients) received a mean transit dose of 395 rads (340–520) and the number of blood volumes passing the radiation field per hour varied from 0 7 to 2.0. Neither in group A nor in group B could a significant correlation be found between the number of blood volumes radiated per hour and the total number of blood volumes radiated until the "base level" of the lymphocyte concentration was reached.

The duration of lymphopenia following cessation of ECIB The lymphocyte concentration after cessation of ECIB was checked weekly in patients who were not transplanted immediately after ECIB and therefore received no additional immunosuppressive therapy Fig. 8 shows the lymphocyte concentration in per cent of pretreatment values in 9 patients who received 400 rads in transit dose followed 14 months after the end of ECIB The concentration remained constant at about 30% of the pretreatment value during the first 8 months. At 12 months it had increased only to about 50%

From Fig. 8 it can also be seen that the lymphocyte concentration in 6 patients who received 100 rads in transit dose had increased to 50% already 3 months after the end of ECIB

Preliminary results in two patients treated in ECIB III The decrease of lymphocyte concentration in the two patients treated in the portable beta unit for about 160 hours, with a transit dose of 19 rads, is presented in Fig. 9 In both patients the lymphocyte concentration decreased to about 1/3 of pretreatment value after about 500 radiated blood volumes. The total radiation dose given was 9 000 and 8 650 rads. After cessation the concentration was followed during the first month and slow increase was observed.

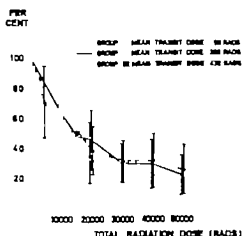


Fig. 6. Lymphocyte concentration in the peripheral blood during ECIB Average values and S.D. Ordinate: lymphocyte concentration in per cent of pretreatment value. Abscissa: total radiation dose (rads).

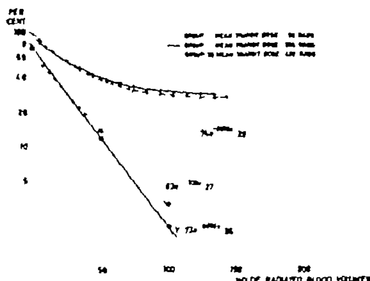


Fig. 7. Lymphocyte concentration in the peripheral blood during ECIB. Ordinate: lymphocyte concentration in per cent of pretreatment value. Abscissa: number of blood volumes radiated. ● = mean values, O = values obtained after subtraction of the right from the left descending part of the upper curve (●).

DISCUSSION

The lymphocytes can be divided into two populations, one dependent on the presence of the thymus (T-lymphocytes) and the other independent of the thymus (B-lymphocytes) (7). They differ in their halflife, their distribution and perhaps in their ability to blast cell transformation (4, 5, 9). The T-lymphocytes constitute the greater part of the recirculating pool of small lymphocytes and are long-lived. The B-lymphocytes are restricted to the lymphoid tissue and are more short-lived. Stimulation with non-specific mitogens such as phytohemagglutinine (PHA), more specific antigens such as purified tuberculin (PPD) and mixed cultures of allogeneic cells (MLC) can induce mitoses in cultured small lymphocytes with transformation to larger (blast) cells. These agents exert their effect predominantly on the T-lymphocytes.

ECIB is generally believed to cause destruction of the T-lymphocytes ( ). In the present study a rapid decrease in the lymphocyte concentration during ECIB (Fig. 7) and a slow increase about 8 months after cessation of ECIB (Fig. 8) were observed. These findings might represent killing of the T-cells followed by reproduction of these cells about 8 months after the end of treatment. The stable concentration of the remaining 30% of the pool (horizontal part of the curves in Figs. 7 and 8) during continuous ECIB and during the first 8 months after cessation might represent the B lymphocytes. On the other hand the results of lymphocyte transformation tests (16) and chromosome markings (6) point to the fact that the ratio between T and B-lymphocytes remained unchanged after ECIB. The lymphocyte response to stimulation with PHA in 20 patients and to stimulation with PPD and allogeneic cells in 8 patients was unchanged per unit number of cells after ECIB (16). Field et al. (6) showed that the lymphocytes left after ECIB are just as radiosensitive as before. The most likely explanation of the findings in the present investigation is therefore that ECIB destroys both the T and B-lymphocytes. The different slopes of the curves in Figs. 7 and 8 might represent destruction of cells from different compartments, i.e. from a rapidly and a

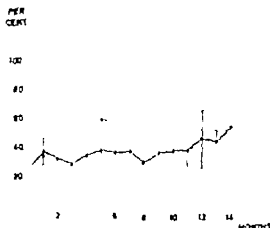


Fig. 8. The lymphocyte concentration after cessation of ECIB. Ordinate: lymphocyte concentration in per cent of pretreatment value. Abscissa: months after the end of irradiation. Mean values and S.D. in 9 patients (average transit dose 395 rads) followed 14 months. Dashed line = mean values in 6 patients (average transit dose 96 rads) followed 6 months.

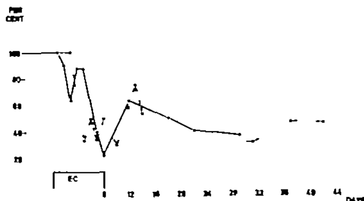


Fig 9 The lymphocyte concentration in two patients continuously treated in the beta units for 15 and 172 hours with transit dose of 19 rads. — — — Patient 1, — — — patient 2. Ordinate: lymphocyte concentration in per cent of pretreatment value. Abscissa: days.

slowly exchangeable pool. According to this one would expect that the curves in Figs. 7 and 8 can be dissolved into two slopes which are both significantly different from zero. With the small number of observations in the present study it was not possible to demonstrate whether the slopes of the apparently horizontal lines in Figs. 7 and 8 were statistically different from zero.

According to Sharpe (14) about 50% of the lymphocytes are killed by a transit dose of about 400 rads. In the two groups of patients (groups II and III) in this study who received a mean transit dose of 359 and 43 rads, the concentration of the lymphocytes from the rapidly exchangeable pool is halved after about 20 radiated blood volumes. The size of this pool would then be about 20 times the lymphocyte pool in the blood, and this is in good agreement with the findings of others (6, 8).

The present study was primarily designed to examine the optimal conditions for ECIB as immunosuppressive measure in transplantations. The following practical conclusions appear relevant. Prolonged reduction of the lymphocyte concentration is obtained with ECIB with transit doses of 300 to 400 rads and total radiation doses of about 50 000 rads. However this schedule causes hemolysis with increased requirement for blood transfusions. If the total radiation dose is reduced below 30 000 rads, by reduction of the transit dose to 100 rads, no measurable hemolysis is caused by the treatment. The degree of lymphopenia is the same as obtained with higher doses. However whereas lymphocyte concentration did not increase until 8 months after cessation of ECIB with the higher total radiation doses, an increase was observed after 3 months with the lower total radiation doses. Therefore the treatment has to be repeated every third month when the low dose is used. This can be effectuated without significant hemolysis. The influence of varying transit doses on the production of lymphopenia on humans in the present study is in accordance with the experimental results on calves of Sipe et al. (15).

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A STROKE REGISTER IN GÖTEBORG, SWEDEN

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Abstract In January 1970 Stroke Register was established in Göteborg, Sweden, covering all cases of acute cerebrovascular incidents occurring in inhabitants of the city born in 1904 and later. This was initiated by the success of the existing Myocardial Infarction Register and constitutes an autonomous but integral part of the Heart Control Programme of Göteborg that has been in progress since 1968 and is partly supported by the WHO The method of registration and the results from the initial 6-month period (Jan. 1st – June 30th, 1970) are presented. From all cases of stroke notified clinically through hospital contact (all acute disease manifestations are traditionally served centrally at the one major hospital of the city) and from cases additionally detected through autopsy recordings and death certificates, various information was collected. This consisted of selection of personal data, previous medical history of cardiovascular diseases, clinical findings on admission, routine laboratory results, and findings at special neurological examinations. The results show total number of 102 registered cases and male/female ratio in these age groups of nearly 2 : 1 The rate of co-existent cardiovascular diseases was found to be high when compared with prevalence data of two running population studies for men and women from Göteborg of similar ages. Other data obtained gave rise to several indications of association with various clinical factors, though the limited numbers of patients do not allow any conclusions so far. It is concluded that the establishment of stroke register seems prerequisite as basic measurement tool in order further to describe the natural history of stroke in community and to assess preventive, therapeutic and rehabilitation measures both for the present hospital-oriented and for implementation of an eventual community stroke control programme.

Cerebrovascular diseases represent major health problem in most communities, industrialized as well as non-industrialized. The incidence of acute cerebrovascular disease or stroke is high The mortality from stroke ranks in most countries as number three in frequency as cause of death in the official vital statistics. Equally important is the high number of severely handicapped persons amongst the survivors of the acute attack.

During recent years community programmes for control of cardiovascular diseases have been launched in a number of places. In most instances the primary aim has been the study and control of ischemic heart disease and of hypertension. WHO has initiated and coordinates some of these activities. The Cardiovascular Disease Unit of the WHO Headquarters thus collects information from 17 registration centres about myocardial infarctions.

In some population studies of ischemic heart disease a knowledge has been obtained about cerebrovascular disease and factors associated with it. This seems especially to be evident for arterial hypertension and blood lipids. The vital statistics concerning causes of death have also added to the knowledge of cerebrovascular disease. This information is, however to a large degree of questionable value. Kurtzke pointed out in his monograph (5) that for stroke a correlation with age only could be established with any degree of certainty

It is thus obvious that there is a need for more and accurate information about cerebrovascular disease concerning morbidity the ways stroke presents in the population, and the frequency distribution of patients by age, sex and previous cardiovascular disease. Information about many of the factors known to operate in relation to the development of atheromatosis is also needed.

At a WHO meeting on Prevention, Treatment and Rehabilitation of Cerebrovascular Diseases, held jointly by WHO Headquarters and its Regional Office for Europe in Monaco in May 1970 some of the following recommendations were made (8)· 1) Prevention of cerebrovascular disease should be attempted by adequate treatment of arterial hypertension. 2) Comprehensive

management should be made available to all stroke patients within practicable limits, including after-care facilities. 3) To assess these activities, follow-up studies were needed to elucidate further the natural history of cerebrovascular disease including more strict therapeutic evaluation.

A possible tool would be pilot studies using cerebrovascular disease registers permitting complete coverage of specified population groups.

MATERIAL AND METHOD

Background. I Göteborg community Heart Control Programme has been successively implemented since Jan. 1968. The emphasis is placed on ischemic heart disease and arterial hypertension. It includes now population studies, primary and secondary prevention trials, health education and follow-up studies of patients with myocardial infarction. As one important underlying instrument of measurement, register of ischemic heart disease was established at an early stage. This register provides accurate information on the occurrence of acute myocardial infarctions in the total community. It gives details of all persons concerned on continuing basis. It fuses clinical research, epidemiology and medical care into single operation serving the present needs of community medicine.

In Jan. 1970 this programme was extended to include Stroke Register. This was brought about by collaboration between Medical Department I, which runs the Heart Control Programme, and the Department of Neurology and quickly obtained the support of WHO. The principles underlying this register are similar to those for the register of ischemic heart disease: (a) to collect information for descriptive epidemiological analysis—to further describe the natural history of stroke, (b) to describe and evaluate the diagnostic and therapeutic measures undertaken both in the acute and in the chronic phase of stroke and (c) possibly to give base line data for cost-benefit analysis.

It appears necessary to establish basic measurement tool such as the register if the magnitude and impact of the stroke problem in the community are to be measured. The real effect of any intervention—preventive diagnostic and or therapeutic—can only be assessed if such baseline data are accurately assembled.

The city of Göteborg has population of nearly half million. The hospital services for the management of acute disease are centralized, and Sahlgren Hospital serves nearly all cases of acute disease in the city.

Initially it was decided to confine the registration to cases of stroke occurring in persons of 65 years and younger. At the outset no attempts were made to deviate from the routines of individual wards and departments in their diagnostic and therapeutic activities. This was, for practical reasons, the most feasible procedure. It would also permit an evaluation of the degree to which diagnostic measures, including laboratory as well as special examinations and history taking, are performed at present. Which items should optionally be implemented in any standardized activity at later stage could be determined later.

Case detection. Notification of stroke-suspected patients in contact with the hospital is performed by a small group headed by a nurse. This group has daily contacts with casualty departments, all medical, neurological and neurosurgical wards, as well as with out-patient departments, EEG laboratory, brain-scan laboratory etc.

Entry in register. All notified patients were either seen by the neurologist or their case histories were examined. All cases of stroke occurring during the period Jan. 1st–June 30th, 1970 in inhabitants of the city born in 1904 or later were included in the registration. Cases which at post-mortem examination showed signs consistent with recent cerebrovascular accidents and cases in which stroke was assigned as the main cause of death on death certificates were also included.

Definition. Stroke has here been defined as rapidly developed clinical signs of focal (and or global) disturbance of cerebral function of presumed vascular origin and of more than a few minutes duration.

Information collected. From each such case information on the following items was sought. When obtainable they were entered into standardized initial record form.

Identification: name, address, occupation, date of birth. The source of information. Date and time of admission or contact with medical services (if any). The length of time from onset of present attack until contact with medical services. The type of care: hospitalization, outpatient care or neither. The duration of hospitalization. Diagnoses at discharge. Destination after discharge: home or institution; if dead, the date and time of death and the findings at post-mortem examination if performed. Personal data: sex, civil status, physical performance during work, smoking habits, habits of alcohol consumption. Family history: if any of the parents had died before the age of 70 from heart disease or stroke, this was noted. Previous medical history: information on myocardial infarction or other heart disease, intermittent claudication, diabetes mellitus, hypertension, and whether this was under treatment prior to the present stroke. The presence of any other non-vascular disease was also recorded. Any previous manifestation of cerebrovascular disease: its type, and the time of the latest attack were noted. Premonitory symptoms: their type, especially those related to the central nervous system, and their duration. Circumstances in conjunction with the onset of the present attack: the place of onset, relation to any physical or mental exercise or strain, and the relation to sleep. Clinical findings at first medical examination: description of the type of neurological deficit, the level of consciousness, the BP, eyeground findings, and any presence of carotid murmurs. Further diagnostic measures: lumbar puncture, whether performed, the time of performance after "contact" (admission) and the findings. If performed, X-ray of skull, echo-encephalography, isotope-encephalography and cerebral angiography were noted, as well as the time and the findings at repeated such examinations. Laboratory findings: values for blood Hb, plasma creatinine, triglycerides

and cholesterol. Furthermore ECG and X-ray f heart and height and eight ere recorded.

T ensure the best possible coverage of all stroke cases, all post mortem records and death certificates in the city related to the registration period were examined, and cases not already entered through the clinical notification ere included. The cases detected in this w y comprised around 15 % f the total number registered, though the amount of detailed information in these cases as small. Furthermore death certificates for the latter half of 1970 ere examined in order to assess the number of late deaths (at 6 mo. after onset) amongst the registered cases surviving the initial hospitalization period.

Data processing. From the standard initial record form selection of ll information collected, which appeared reasonably well represented, was transferred to punch cards, and all qualitative data ere xtracted with card sorter machine for presentation in tables and figures; quantitative data (mean values) were calculated on desk computer.

RESULTS

The total number of cases registered between Jan. 1st – June 30th, 1970 was 102. This gives an incidence of 52/100 000 population and year for the age group of 65 years and younger

A presentation of the material is given in Fig. 1 The lower boxes give the distribution in diagnostic groups based on clinical and/or autopsy findings. The initial mortality (during the initial hospital stay) for each diagnostic group is also shown. The upper boxes give an assay of the prognosis for all cases. The five cases of death outside hospital consisted of two men and three women all above 53 years of age four had subarachnoid hemorrhage (SAH) and one intracerebral hemorrhage discovered at autopsy About one third of the patients died during the initial hospital stay The length of this varied to a large degree, the mean being around 26 days. Two pa-

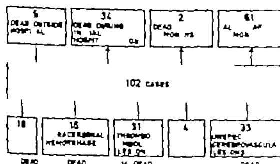


Fig 1 Diagnoses and follow-up results. Goteborg Stroke Register.

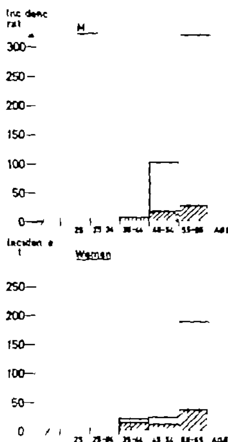


Fig 2 Distribution of SAH ( ) and other stroke cases ( ) by sex and age groups. Incidence rates are given per 100 000 population and year for each sex and age group

tients were discharged alive but died within six months of the onset of stroke the rest (60 %) survived more than six months Two patients were treated on an out-patient basis only 95 were hospitalized

The distribution for males and females by age appears in Fig. 2, calculated as incidence (number per 100 000 population and yea for each age group and sex, respectively). The overall numbers increase with age but it also appears that there is preponderance of male stroke cases over female This seems to be so particularly in the younger age group (45–54 y) for stroke cases other than SAH. The total male/female ratio is nearly 2 1 (65 male and 37 female cases)

Fig. 3 illustrates the time from onset of symptoms until contact with medical services in cases where this information was obtained. The great

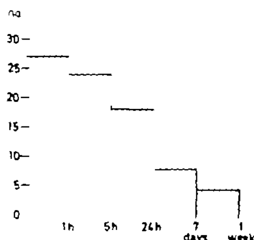


Fig 3 Time from onset of symptoms until contact with medical services *N* 60

majority of patients came under medical care within the first 24 hours, only very few appeared after one week.

Analysis of the information obtained about circumstances in conjunction with onset of symptoms showed that the vast majority of onsets occurred at home Six patients only were at their work at onset and these were all men In seven cases onset occurred in traffic (walking or driving) A considerable proportion (17 cases) had their onset of symptoms while in hospital or in other institution

When considering the relation onset of stroke to sleep, it was apparent that the large majority experienced their first symptoms during normal day-time activities (no relation) only a few occurred during sleep ("woke up with" or were woken up by" symptoms) An initial suspicion that the first few hours after awakening were particularly related to the occurrence of stroke symptoms was not substantiated when more cases were collected.

Table 1 presents the clinical findings at first medical examination. This information is largely consistent with similar reports by others a slightly larger proportion of right-sided hemidisorders compared with left-sided. Other focal symptoms comprise signs of brain stem lesions and left plus right sided hemidisorders. There does not seem to be any tendency to a difference in initial mortality relating to the type of deficit, though no deficit most often comprised cases with SAH A special group is the one with missing data for which no information was obtainable about clinical findings on admission The reason for the missing information in this group is inherent in the initial method of registration. In part this is related to the fact that all of these patients died (often within a short time). The frequency of dysphasia found on admission was 31

In Table 1 the patients are also grouped according to level of consciousness on admission and to the relation to diagnosis and initial prognosis. Around 60 % of the patients were fully awake on arrival in hospital. The distribu-

Table 1 *Clinical findings at first medical examination (type of neurological deficit and level of consciousness) and initial mortality*

	No of pat	Cerebrovascular diagnosis		Discharged	
		SAH	Other strokes	Alive	Dead
Neurological deficit					
No deficit	9	7	2	3	6
Right hemidisorder	39	0	39	28	11
Left hemidisorder	34	5	29	28	6
Other focal signs	6	2	4	4	
Missing data	9	0	9	0	9
Level of consciousness					
Awake	56	4	52	49	7
Somnolent	20	6	14	11	9
Semicoma		0	2	0	
Coma	15	4	11	3	12
Missing data	4	0	4	0	4
Total no of hospital cases	97	14	83	63	34

tion between diagnostic groups does not show any special trends. There was, as expected, a pronounced increase in mortality among the patients admitted in comatose condition.

Some diagnostic measures are listed in Table II with the number of cases in which they had been performed. Sometimes they might be characterized as special examinations and would not have been carried out unless clinical indications were present. To some extent this table also reflects the extent of diagnostic activity

In hospitalized cases, surviving more than one hour and clinically suspected of stroke a spinal tap had been performed in 60%. Amongst the 46 patients in whom no spinal tap was done nine were not suspected clinically of stroke and one died within one hour after admission.

The diagnostic group to which a stroke case was referred (and here autopsy diagnoses are also included) depended to a large extent upon whether cerebrospinal fluid was examined or not (Table III). Information on this item was lacking in 4 cases.

The presence of other cardiovascular diseases in the patients with stroke and the frequency of

Table II. *Diagnostic measure performed in 97 strok cases served at hospital (information not retrieved in 4 cases)*

	Performed	Not performed
Lumbar puncture	47	46
X-ray of skull	45	48
Echo-encephalography	58	35
Isotope-encephalography	17	76
Cerebral angiography	33	60

Table III. *Relation of lumbar puncture t diagnostic group (hospital served cases)*

	Performed	Not performed
SAH	13	1
Intracerebral hemorrhage	9	6
Thrombo-embolic lesion	15	13
TIA only		2
Unspecified cerebro-vascular lesion	8	24
Total no.	47	46

Table IV *Co-existent and previous diseases in stroke cases*

	Men		Men born 1913	Women		Women study
	( )	( )	( )	(n)	( )	( )
Heart disease	27	43	6.4	10	29	5.3
Hypertension	30	48	7.8	16	47	10.9
Diabetes	8	13	0.8	3	9	1
Previous cerebro-vascular disease	22	35	0.3	8	23	—

previous cerebrovascular disease are shown in Table IV The values for 65 men and 37 women are listed together with prevalence values from the two population studies from Goteborg: Men born 1913 (at follow-up examination in 1967) (7) and the "Women study" (1) the age groups of which are both 54 years. As the mean age for the male stroke patients was 57 years and for the female patients 58 years, this permits a fairly adequate comparison The stroke patients had a markedly increased frequency of heart diseases (the vast majority being ischemic) hypertension and diabetes. This difference is more pronounced in the male patients, being 5–10 times the prevalence in the population. Of the 19 male patients with previous cerebrovascular disease ten had had this in the form of transient ischemic attacks (TIA) only In the female patients this was so in only one case Other non-vascular diseases were represented with a high frequency in the stroke patients (around 50%). Adequate control values from the population studies are not available These diseases represented all organ systems, most often respiratory gastro-intestinal and kidney diseases Around one third of the men, a smaller proportion of women, had more than one of these non-vascular diseases. Alcoholism was present relatively often.

Regarding the causes of death of the patients parents, stroke was more often the cause in one parent of the female patients, whereas no clear cut impression regarding the male stroke patients appeared (Table V) Information on these items was often not obtainable.

The duration of stay in hospital was not related to the diagnostic group. If duration of hospitalization was short, this was because the patient died. The mean duration of hospitalization was 27 days



experienced their first symptoms during normal day time activities (no relation), only a few occurred during sleep ("woke up with or were woken up by symptoms). An initial suspicion that the first few hours after awakening were particularly related to the occurrence of stroke symptoms was not substantiated when more cases were collected.

Table I presents the clinical findings at first medical examination. This information is largely consistent with similar reports by others: a slightly larger proportion of right-sided hemidisorders compared with left-sided. Other focal symptoms comprise signs of brain stem lesions and left- plus right-sided hemidisorders. There does not seem to be any tendency to a difference in initial mortality relating to the type of deficit, though "no deficit" most often comprised cases with SAH. A special group is the one with missing data for which no information was obtainable about clinical findings on admission. The reason for the missing information in this group is inherent in the initial method of registration. In part this is related to the fact that all of these patients died (often within a short time). The frequency of dysphasia found on admission was 31%.

In Table I the patients are also grouped according to level of consciousness on admission and to the relation to diagnosis and initial prognosis. Around 60% of the patients were fully awake on arrival in hospital. The distribu-

I ... lon (type of neurological deficit and level of consciousness)

		C ov cular diagnosis		Discharged	
		SAH	Other strokes	Alive	Dead
N... ...					
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Right hemidisorder	39	0	39	28	11
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Missing data	9	0	9	0	9
Level of consciousness					
Awake	56	4	52	49	7
Somnolent	20	6	14	11	9
Semicoma		0	2	0	2
Coma	15	4	11	3	12
Missing data	4	0	4	0	4
Total no. of hospital cases	97	14	83	63	34

Table VIII. *ECG and cerebrovascular diagnosis of 97 hospital-served cases*

		Cerebrovascular diagnosis				
ECG	Total no.	SAH	Intra-cerebral hemorrh.	Thrombo-embolic lesion	TIA only	Unspecified cerebrovascular lesion
Normal	38	4	4	12	0	18
Signs of left ventr dominance and/or ischemia	35	5	10	10	1	9
Fibrillation or flutter	7	0	0	4	1	2
Not performed	12	4	1	3	2	2
Unknown	5	1	0	2	0	2
Total no.	97					

Hospital case histories usually contain a vast amount of information. A standardized selection of this information has primarily been chosen. Although nearly none of the results presented in this study give any clearly unquestionable answers, many indications or even suspicions of associations obtained from analysis of the information justify the continued collection of information on these lines.

It may be argued that the case detection procedure may be far from covering all patients with stroke in the community even in the age groups here concerned. The local routine is, however that all patients with acute disease manifestations come into immediate contact with the central medical service provided by Sahlgren's Hospital. This would especially be the case with younger persons. There is thus reason to believe that almost all stroke cases have come to our knowledge

This is further supported through the present Preventive Trial in Göteborg, in which so far 2 500 men born in 1915–19 have been screened for cardiovascular disease. Among this number 12 subjects had had a previous stroke and all of them had been hospitalized at the time of the stroke. Case detection control within the hospital has also shown that, of cases admitted, almost all were reported. A separate study however of the frequency of treatment of stroke particularly minor attacks, in general practice without any hospital contact is planned and will soon be performed.

The method of data collection is also of crucial importance. This concerns both the construction of the questions in the record forms and the answering of them when faced with the stroke patient. In this study two persons have been responsible for filling in the forms. All doubtful questions have been discussed in detail in order to obtain the necessary uniformity

The reason for confining the registration of stroke cases in the present pilot study to persons of 65 years and younger was partly to limit the work load and partly because any associated factors might reasonably be expected to appear more clearly in the younger age groups. The diagnostic problems are also easier to establish in younger persons, as older individuals often suffer from a number of diseases of which stroke may be only one.

In 1963 Broman and Lindberg-Broman (2) found that stroke cases occurring in Goteborg in persons of 65 years of age and younger comprised around 30% of the total number The incidence found in the present study 52/100 000 and year for persons 65 years and younger would thus give an estimated total incidence for stroke in Göteborg of around 150/100 000 population and year Broman and Lindberg-Broman in their study furthermore, found an incidence for SAH somewhat lower than the present figure. This might be explained by the confinement of this study to the younger age groups only

The present finding of a high male/female ratio is consistent with the reports of others (3, 4 6) in series of younger patients admitted to neurological or neurosurgical departments and with angiographically demonstrable occlusions within the carotid system. The selection criteria for these series are, however not clearly stated.

Our finding of a high frequency of other diseases than stroke, both vascular and non-vas-

cular is striking. This indicates that most of the stroke patients already have vascular or non-vascular diseases.

Displeasingly large figures appear for items concerning which no information was obtainable by the methods used. This evidently calls for a different approach, with better specified programmes concerning both registration of the medical history and routine laboratory examinations, including lumbar puncture and ECG One consequence may be that routine activities in individual wards will have to be interfered with to some degree.

Concerning the more special examinations, such as cerebral angiography there is no reason to use them on a wider scale than hitherto as they should be performed on clinical indications only

At the WHO meeting in May 1970 on the Prevention, Treatment and Rehabilitation of Cerebrovascular Diseases (8) studies were considered desirable which might uncover factors associated with stroke— risk factors"—and provide better morbidity statistics and validate mortality statistics. Studies on evaluation of treatment of the acute phase of stroke as well as of rehabilitation during the chronic phase were also requested. The establishment of stroke registers was considered an important and useful way of approaching these questions

The present pilot study shows that simple registers of new cases of stroke patients is not enough to meet these recommendations. The methods of registration must also be improved. Work is at present being carried out in this respect. An extended "initial recording form covering the first 21 days after onset is now in use, giving more and uniform details of the patients histories and from repeated clinical examinations. It includes also a laboratory examination list of simpler tests to be followed in all cases. The experience gained might provide further data of importance for the final design of record forms to be worked out under WHO auspices for more general use.

The next step concerns follow-up examinations at regular intervals in order to collect information on the long-term clinical course and to evaluate rehabilitation activities and the social circumstances of the stroke patient. Data are to be collected especially through these follow-up examinations in order to evaluate the possible desirability of establishing a special out-patient after-care unit for stroke patients. Of special interest seems to be the design of an easily handled clinical evaluation system covering both neurological impairment and social disability A simple but accurate scoring system would improve the future possibilities of evaluation of different diagnostic, therapeutic and rehabilitation measures.

ACKNOWLEDGEMENT

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PENTAGASTRIN AND INSULIN INDUCED SECRETION IN DIABETES MELLITUS

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Abstract. The insulin test has been accepted as the most reliable means of evaluating vagal function. A modified form of n—combined insulin/pentagastrin test—demonstrated in a controlled study that the vagal influence on gastric secretion is reduced, suggesting vagal dysfunction in 10 diabetics with an average duration of diabetes of 15 years without symptoms from the gastro-intestinal tract. This agrees with the contention that delayed gastric emptying is another sign of vagal dysfunction.

Gastro-intestinal disturbances are part of the clinical picture of diabetes mellitus, not only in association with ketoacidosis. Rundles (12) described four patients with constipation and diarrhoea and roentgenological evidence of gastric retention. He emphasized that gastro-intestinal disturbances often accompany diabetic neuropathy and that these symptoms are an expression of disease of the autonomic nervous system. Kassander (9) proposed the concept gastro-paresis diabeticorum. In six diabetic patients without symptoms from the gastro-intestinal tract he found a more or less atonic stomach with delayed emptying on X-ray examination. Dotevall (6) showed by saline test meals that delayed emptying occurs in diabetic patients with late complications. The rate of gastric emptying was measured by Fagerberg (7) by a method in which the stomach is scanned at intervals after a standard breakfast containing Cr. He demonstrated that diabetic patients with long duration of the disease and with severe diabetic complications had delayed gastric emptying. The clinical, roentgenological and experimental findings of gastric dysfunction suggest a lesion of the vagus nerve. Very few investigations have been performed on pathological findings in the autonomic nervous system of diabetic patients. Kristensson et al. (10) report changes in the vagal nerves in some diabetic patients with long duration. Hensley and Soergel (8) investigated three diabetic patients with regard to neuropathological changes. Abnormal findings were seen in prevertebral and paravertebral ganglia but not in the vagal nerves.

The ability of insulin to stimulate gastric secretion depends on its hypoglycaemic activity. Cerebral hypoglycaemia stimulates the vagus centre, which in turn excites the gastric glands by way of the vagus nerve. The purpose of the present investigation was to study the effect of insulin hypoglycaemia compared to pentagastrin on gastric secretion in patients with diabetes mellitus, and in this way to investigate the vagal function.

MATERIAL AND METHODS

The investigation was performed on 10 male diabetic patients with varying duration of diabetes (mean duration 15.0 ± 8.1 y; means ± S.D.) and on 10 controls. The diabetic patients and the control subjects were comparable in age, averaging 29.3 ± 7.0 years (means ± S.D.) against 28.9 ± 6.4. The age distribution is given in Table I. All the diabetic patients were on insulin and prescribed diet consisting of 50% carbohydrates, 20% proteins and 30% fats. All patients were in a clinically satisfactory metabolic state. The control subjects revealed no dietary peculiarities and were apparently healthy.

Clinical methods

All patients were examined routinely. The following diabetic manifestations were examined.

1. Retinopathy. Ophthalmoscopy was performed at the Department of Ophthalmology of the hospital. Retinopathy was graded as follows: 0 = no changes, 1+ = microaneurysms, + = microaneurysms and haemorrhages with or without exudate, 3+ = proliferative changes.

Table I *Clinical data on the patients*

Case no.	Age (y.)	Duration of diabetes (y.)	Retinopathy	Nephropathy	Neuropathy
1	21	13	3+	+	+
2	23	6	3+	+	+
3	23	7	0	0	0
4	26	10	0	0	0
5	27	24	1+	+	+
6	27	20	1+	+	+
7	33	14	0	+	0
8	33	19	3+	+	+
9	39	7	3+	+	+
10	41	30	1+	+	+

2. Nephropathy. Constant proteinuria without signs of infection (negative bacterial urine culture) was attributed to diabetic nephropathy.

3. Neuropathy. Besides routine neurological examination, electromyography (EMG) was carried out. Diabetic neuropathy was diagnosed on at least two typical neurological signs (e.g. bilateral Achilles areflexia, abnormal EMG).

Clinical data on the patients are given in Table I.

Procedure and laboratory methods

Gastric secretory studies were performed in the morning after twelve hours' fasting and abstention from smoking. A nasogastric tube (Salem Sump Tube) was introduced. The patient was in semisupine position and was instructed not to swallow saliva. Continuous drainage of the stomach was carried out by suction with pump giving subatmospheric pressure of 50 mmHg intermittently. Injection of air and intermittent suction by means of a syringe was used to prevent blocking of the tube. Gastric juice was pooled for individual 15 min periods.

The volume of gastric juice was measured in ml, the pH recorded and the concentration of acid determined by titration with N/10 NaOH to pH 7.0, measured in a pH meter. The acid titrated was taken as hydrochloric acid and its output in mEq/15 min was calculated as product of the volume (l) and concentration (mEq/l). The maximal response to pentagastrin and insulin is expressed as peak acid output (PAO) using the two highest consecutive samples of 15 min converted to mEq/h.

The following tests were performed:

Pentagastrin test. Basal aspirates were collected during one hour. Thereafter a synthetic pentapeptid—pentagastrin (ICI, England)—was given subcutaneously in dosage of 6 μg/kg b.wt. Further collection of aspirates was performed during another hour.

Insulin test. Before the study all patients recived their

Table II *Peak acid output, its volume and concentration for the diabetic patients and the control subjects*

Case no.	Peak acid output (mEq/h)		Volume (ml)		Concentration (mEq H⁺/l)	
	Insulin	Pentagastrin	Insulin	Pentagastrin	Insulin	Pentagastrin
Diabetic patients						
1	39.1	55.7	310	484	126	115
2	1.7	27.1	30	200	57	136
3	29.8	41.0	244	276	122	149
4	27.0	45.1	240	352	113	128
5	25.1	29.3	244	224	103	131
6	0	0.5	8	48	0	10
7	27.8	51.5	290	410	96	126
8	24.2	38.8	270	396	90	98
9	21.2	36.1	228	346	93	104
10	2.5	15.6	86	174	29	90
Mean	19.8	34.1	195.0	291.0	82.9	108.7
S.D.	13.6	16.7	110.5	131.2	41.4	39.2
Control subjects						
11	26.8	23.8	224	202	120	118
12	27.9	42.1	224	408	125	103
13	30.2	37.8	276	292	109	130
14	38.4	32.7	330	264	116	124
15	36.8	31.8	316	264	116	121
16	25.6	33.4	196	334	131	100
17	20.1	35.9	164	272	123	132
18	14.5	23.8	140	290	104	82
19	38.3	39.4	292	334	131	122
20	28.6	33.8	242	316	118	107
Mean	28.7	33.5	240.4	296.6	119.3	113.9
S.D.	7.8	6.0	63.3	54.4	8.7	15.6

regular dose of insulin. When the gastric secretory studies started, samples of capillary blood were obtained for blood sugar determination by the glucose-oxidase method (11). In order to get immediate rough information, blood sugar as pproximated with the aid f test strips (Dextrostix, Ames, England), which were used simultaneously ith the glucose-oxidase method. After 60 min collection of basal secretion and determination of blood sugar 20 I.U. of insulin (Insulin, Vitrum, Stockholm, Sweden) was given I. to the control subjects. Between 16–32 I.U. was given to the diabetic patients depending on the initial blood sugar value. The secretion as followed for tw hours and blood sugar determined every 30 min. Clinical signs of hypoglycaemia ere seen in all patients and control subjects. All diabetic patients and control subjects ho did not attai blood sugar level at least as low as 40 mg/100 ml after insulin administration were excluded from the study

Conventional statistical methods have been used Student' *t*-test and Wilcoxon test for testing the significance of differences between the groups. A *p* value of <0.05 is considered significant.

RESULTS

Pentagastrin test As seen in Table II, the secre tory response to pentagastrin measured in mean peak acid output, its volume and hydrogen concentration for the diabetic patients were almost identical with those for the control subjects. No significant differences were noted.

Insulin test The mean response to insulin of the diabetic group with respect to hydrogen concentration was significantly lower than that of the control subjects ($p<0.0125$). The mean volume of gastric juice was lower among the diabetic patients than among the controls, though the differ

Table III. *The ratio of the insulin response to the pentagastrin response (PAO_I/PAO_{pg}) for the diabetic patients and the control subjects*

Case no	Patients (%)	Case no.	Controls ( )
1	70.2	11	112.6
2	6.3	12	66.3
3	72.7	13	79.9
4	39.9	14	117.4
5	85.7	15	115.7
6	0	16	76.6
7	54.0	17	56.0
8	62.4	18	60.9
9	58.7	19	97.2
10	16.0	20	84.6
Mean	48.6		86.7
S.D.	30.0		23.0

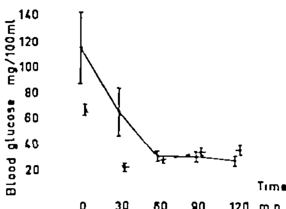


Fig. 1. Blood sugar levels (mean ±S.E.) for the diabetic patients (—) and for the control subjects (– –) before and during insulin hypoglycaemia.

ence was not significant. Hence the a erage acid output for the diabetic group was significantly lower than that for the control group ($p<0.05$).

Combined insulin/pentagastrin test. The mean ratio of the insulin response to the pentagastrin response (PAO_I PAO_{pg}) was calculated for each person (Table III). The mean ratio for the diabetic group was highly significantly low r than that for the control subjects ($p<0.01$)

The mean blood sugar levels in both groups before and after insulin injection are compared in Fig. 1. The mean initial blood sugar alue for the diabetic patients was significantly higher than for the control subjects. This difference had diminished half an hour after the insulin injection. After an hour the corresponding levels were prac tically equal. After an hour and a half the figures were still almost equal. After two hours, how ever the groups were again unequal, the diabetic patients being harder pressed than the controls.

DISCUSSION

The insulin test is well established. It has been constructed and adapted for duodenal ulcer patients pre and postoperatively. The object of the test is to get reliable criteria of the completeness of gastric vagotomy a positive response denoting intact vagus and a negati answer complete vagotomy. Bachrach (2) found that the postoperative response to hypoglycaemia was not an all-or-none response but proposed that the higher the rise in acid secretion after insulin administration the

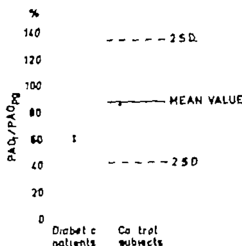


Fig. 2. Combined insulin/pentagastrin test for the control subjects (mean ±2 S.D.) compared with the diabetic patients.

more parasympathetic nerve fibres remained. A weak vagal response to insulin may be explained by low secretory capacity which is revealed by parallel low response to direct humoral stimulation by pentagastrin. Baron (4) proposed a quantitative measure of the vagal activity for intact functioning vagus the ratio of the vagal secretory response to the pentagastrin-stimulated response ranges from 45–165% which is in agreement with the ratio of the present control subjects—the mean ratio ±2 S.D. was 87±46% which ves the range 41–133%.

Earlie workers (1) have ascribed to the diabetic condition high incidences of achlorhydria and low gastric secretion after maximal histamine stimulation. However in our series of diabetic patients the mean PAO after pentagastrin administration was well nigh identical to the mean PAO of the control subjects, and the mean PAO in each group was high compared with normal values within age sex and body weight groups (5). On the other hand the mean vagal response of the diabetic patients was much weaker than that of the controls. A single diabetic patient had an insulin/pentagastrin response ratio attaining the mean level of the control subjects. The remaining nine patients lay below this mean of 86.7% (Fig. 2). Baron (4) proposed for completeness of surgical vagotomy a PAO /PAO_{pg} of ≤40%. Venables and Johnston (13) found in all subjects with surgically proven incomplete vagotomies a peak insulin/pentagastrin ratio of over 50% for acid. Three of our diabetic patients had secretory responses in the range for patients with complete surgical vagotomy. Owing to low secretory capacity in one of the diabetic patients, his insulin response was hard to evaluate. These patients had diabetes of long duration and grave late complications. On the other hand other patients with equally grave late complications of diabetes showed a normal insulin/pentagastrin ratio. This apparent discrepancy indicates that late complications of diabetes have a patchy distribution.

The response of gastric secretion to insulin-induced hypoglycaemia is generally considered to be an all-or-none phenomenon. It is generally accepted that a blood sugar level below approximately 50 mg/100 ml elicits a maximal secretory response. This value has to be reduced to about 40 mg/100 ml with the method used in this study to determine the blood sugar. In none of the diabetic patients did the secretion increase when the blood sugar was more than 40 mg/100 ml.

The hypothesis that the blood sugar must reach a certain level before stimulation of acid secretion will begin seems also true for diabetic patients. In recents years, however this view has been criticized mainly by Baron (3). He suggests that the absolute blood sugar level is not the sole secretory stimulus, that the fall itself and the rate of fall are additional stimuli. Moreover a large decline in blood sugar level and absolute levels below 15 mg/100 ml would induce inhibition.

In the present investigation the differences between the blood sugar levels of diabetic patients and controls were not of an order sufficient to affect the results in any decisive manner. In this study only three patients fulfilled the criteria for a negative insulin test, which does not mean that the rest of the patients had a totally functioning vagus. Three of the remaining seven patients had a reduced ratio for PAO_I/PAO_{pg}, which suggests a reduced function of the vagus nerve.

The literature is very sparse on pathological changes in this part of the autonomic nervous system in diabetic patients. Kristensson et al. (10) describe patho-anatomical autopsy findings in the upper vagus nerve region in three cases of diabetes mellitus, in the form of segmental destruction of the myelin sheaths, alteration of the endoneural connective tissues and diabetic angiopathy in and around the vagus nerve.

In a series of studies on diabetic diarrhoea in cases exhibiting no pathological changes other authors discuss the association between autonomic nervous system dysfunction and enteropathy. Kassander (9) and Rundles (12) considered disturbed vagal function as an aetiological factor in both delayed gastric emptying and diabetic diarrhoea, the latter clinically resembling postvagotomy diarrhoea.

The insulin test has been generally accepted as the most reliable means of studying vagal function. The diabetic patients in the present study with mean duration of the disease of 15 years and signs of vascular and neurological involvement—all of whom lacked symptoms from the gastro-intestinal tract and from the autonomic nervous system as a whole—displayed a distinctly reduced influence from the vagal system, manifested by a reduced PAO_I/PAO_{pg} in response to insulin hypoglycaemia and pentagastrin. This finding agrees with the view that delayed gastric emptying is a sequel to a nervous lesion.

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THE EFFECTS OF UNSATURATED AND SATURATED DIETARY FATS ON PLASMA CHOLESTEROL, PHOSPHOLIPIDS AND LECITHIN: CHOLESTEROL ACYLTRANSFERASE ACTIVITY

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Abstract. The plasma concentrations of cholesterol, individual phospholipids and the activity of plasma lecithin: cholesterol acyltransferase (LCAT) have been examined in two groups of healthy subjects on different diets. The diets were isocaloric with 40% fat. One group received a diet rich in unsaturated fatty acids, in which soybean oil was the fat source. The other group was given a saturated fatty acid diet with medium chain triglycerides (MCT). A significant fall in the plasma concentrations of cholesterol and lecithin was observed after 21 days' use of the soybean oil diet. Concomitantly, a significant reduction in the plasma LCAT activity also took place. In the control group on MCT diet no significant changes in plasma lipid concentrations or LCAT activity were noted. The influence of dietary changes on plasma LCAT activity is discussed.

It has long been established that unsaturated fats regularly lower plasma cholesterol and phospholipid concentrations. This effect has justified the use of unsaturated fats in the treatment of hypercholesterolemic states. The mechanism of this effect is, however, still unknown. Recent studies indicate that unsaturated fats cause a redistribution of cholesterol between plasma and tissue pools (10). A study of Frantz and Carey (5) showed that the content of cholesterol in liver decreased in patients fed an unsaturated diet, indicating that other tissue pools must be involved.

Plasma lecithin: cholesterol acyltransferase (LCAT) transfers fatty acids from the lecithin to the free cholesterol of plasma lipoproteins, preferentially the plasma high density lipoproteins (9). This enzyme is an important factor in plasma cholesterol ester and lysolecithin formation and thus plays an essential role in cholesterol homeostasis. Studies in essential fatty acid deficient rats have shown increased LCAT activity in plasma both in vitro and in vivo (17), indicating that dietary fatty acids may interfere with the enzyme activity.

The present study deals with LCAT activity and concentrations of plasma cholesterol and phospholipids in two groups of healthy subjects given unsaturated and saturated fat diets for 21 days.

MATERIAL AND METHODS

Ten subjects, aged 13 to 43 years, participated in the dietary study. The subjects were randomly divided into two groups, each of five persons. During a period of 21 days they received all their meals at the dietary kitchen in the hospital. During the study all tests were run on paired subjects, one on each diet. Each subject represented his own control.

Diet. Two diets were used in this study (Table I). They were isocaloric and contained 40% fat. The only difference between the diets was that the fat source in one was soybean oil and in the other oleum vegetabile tenue, which is medium chain triglycerides (MCT). The soybean oil had an iodine value of 133 and the MCT oil an iodine value of 4. The oils were incorporated in the diet by preparation of "oil-butter" and sauces.

Blood collection. Venous blood was collected after the subjects had been fasting for 14 hours, at the start and at the end of the dietary period. Serum was prepared and stored at -20°C until it was tested.

Lipid analyses. Cholesterol and cholesterol ester were measured by a gas chromatographic method using 5-α-cholestane as internal standard (12). Phospholipids were determined after lipid extraction by a colorimetric method (4). Phospholipid distribution was determined by thin layer chromatography (TLC) on *Silica Gel G* (Merck), 0.3 mm thick, in chloroform-methanol-acetic acid-water 25:15:4:2 according to Skipski et al. (15). The lipid spots were made visible by iodine vapour and scraped off. After hydrolysis of the phospholipids the lipid phosphorus was measured as described earlier (7). LCAT activity in plasma was determined as described by Stokke and Norum (16).

Table I *Composition of experimental soybean oil and MCT oil diets*

	(g)	Protein (g)	Fat (g)	Carbo-hydrate (g)	Kcal.
Breakfast and evening meal					
Bread	230	22	3	120	594
Lean meat	60	12	1.2		60
Skimmed milk	300	10.5	0.3	15	105
Dried skimmed milk	10	3.7	0.1	5.3	37
Jelly	60	0.4		24	97
Sugar	5			5	20
Puffed wheat	25	1.4	0.3	22	97
Dinner					
Lean meat or	205	35	3.3		205
Lean fish	250				
Potatoes	150	2.7	0.1	25	115
Vegetables	150	1.5	0.[illegible]	10	60
Wheat flour	10	1	0.1	7	34
Fruit	150	0.6	0.1	30	120
Additional fat					
Soybean oil or MCT oil[a]	112		112		1 000
Total		98.8	121.5	263.3	2 548

Oleum vegetabile tenue Dansk Soyakage Fabrik, Copenhagen, Denmark.

RESULTS

Table II shows that there was a significant fall in plasma total cholesterol after a 21-day period on soybean oil diet, as mean cholesterol was reduced from 219 mg% to 183 mg%. The percentage of plasma cholesterol esterification was unchanged, 76%. It is further seen that there also was a significant fall in total phospholipids in those on a soybean oil diet. Plasma LCAT activity was significantly reduced from a mean of 47 to 35 µmoles free cholesterol esterified per hour per litre.

Table III shows that a small, but insignificant fall in plasma cholesterol took place during a 3-week period on MCT oil diet. No significant changes were observed in the degree of cholesterol esterification or LCAT activity.

Table IV shows that the fall in total phospholipids during the period on soybean oil diet was mainly due to a significant reduction of the concentrations of plasma lecithin. The small reductions in the other phospholipid fractions were not significant. Table IV further shows that no significant alterations in the plasma phospholipid pattern took place during the 21-day period on MCT oil diet.

Table II. *Total cholesterol, percentage of cholesterol ester, total phospholipids and LCAT activity in plasma from 5 subjects before and after a 21-day period on soybean oil diet*

Subject no	Sex	Total cholesterol (mg%) Before	After	Cholesterol ester (%) Before	After	Total phospholipids (mg%) Before	After	LCAT activity (µmol/l/h) Before	After
1	♂	189	153	77	75	224	140	47	40
2	♀	209	160	76	76	214	162	48	35
3	♂	187	206	78	79	193	136	45	29
4	♂	288	229	73	74	224	179	42	23
5	♂	223	168	75	76	219	165	53	29
Average		219	183	76	76	215	156	47	35
Significance of difference		$p = 0.05$		n.s.		$p < 0.01$		$p < 0.01$	

Table III *Total cholesterol percentage of cholesterol ester total phospholipids and LCAT activity in plasma from 5 subjects before and after a 21-day period on MCT oil diet*

Subject no	Sex	Total cholesterol (mg %)		Cholesterol ester ( )		Total phospholipids (mg %)		LCAT activity (μmol/1/h)	
		Before	After	Before	After	Before	After	Before	After
6	♂	273	244	69	73	275	266	35	36
7	♂	[illegible]20	178	77	73	228	136	69	56
8	♂	203	223	74	75	214	219	59	53
9	♀	298	223	78	75	270	231	62	76
10	♀	213	209	76	75	197	224	42	70
Average		41	215	74	74	237	215	53	58
Significance of difference		n.s.		n.s.		n.s.		n.s.	

DISCUSSION

The present dietary studies in humans have shown that the reduction of plasma cholesterol and phospholipids obtained by a diet rich in unsaturated fats is associated with a significant reduction of plasma LCAT activity The plasma cholesterol ester/total cholesterol ratio remained constant, indicating that this ratio is not solely determined by the rate of plasma cholesterol esterification. The total plasma phospholipid concentration also showed a reduction on an unsaturated fat diet, and analysis of the individual phospholipid fractions unvelled significant reduction in the lecithin concentrations only

In patients given a diet rich in saturated medium chain fatty acids no significant change in LCAT activity was found. It should be emphasized that this diet was not completely deficient in essential fatty acids.

Previous dietary studies in rats have demonstrated increased LCAT activity in plasma of animals fed an essential fatty acid (EFA) deficient diet and in fasted animals (17 18) Animals fed an EFA deficient diet also have increased amounts of cholesterol esters in the liver associated with a lowered plasma cholesterol and an esterified total cholesterol ratio which was unaffected (1).

These studies clearly show that the quantity and the quality of dietary fats influence LCAT activity The present observations of reduced rate of plasma cholesterol esterification on an unsaturated fat diet may reflect a reduced syn-

Table IV *Plasma phospholipid fractions in relative and absolute amounts before and after 21-day periods on soybean oil and MCT oil diets*

LL = lysolecithin, S = sphingomyelin, L = lecithin, PS = phosphatidyl serine, PE = phosphatidyl ethanolamine

	LL		S		L		PS		PE	
	% of total lipid P	mg	% of total lipid P	mg	% of total lipid P	mg	% of total lipid P	mg %	% of total lipid P	mg
Soybean oil diet										
Before	6.0	12.9	16.6	35.7	71.9	154.2	2.0	4.3	3.5	7.5
After	6.3	9.8	19.7	30.7	67.6	105.6	2.4	3.7	3.9	6.1
Difference	+0.3	−3.1	+3.1	−5.0	−4.3	−48.6	+0.4	−0.6	−0.4	−1.4
Significance of difference		n.s		n.s		p<0.01				n.s
MCT oil diet										
Before	7.2	17.0	16.9	40.1	67.4	159.6	4.6	10.9	3.8	9.0
After	8.8	18.9	18.5	39.8	65.1	139.8	2.9	6.2	4.7	10.1
Difference	+1.6	+1.9	+1.6	−0.3	−2.3	−19.8	−1.7	−4.7	+0.9	+1.1
Significance of difference				n.s		n.s		n.s		

thesis or a reduced release of the LCAT enzyme. They may further reflect changes in the substrates for the reaction particularly the principal one, plasma high density lipoprotein. It has already been suggested that plasma LCAT activity in humans correlates with the level of plasma unesterified cholesterol (11). The present observations and earlier ones in animals (17 18) do not support this suggestion. At present no definite explanation can be given of the mechanism whereby dietary fats influences LCAT activity

Grundy and Ahrens (10) have recently suggested that feeding of unsaturated dietary fats in humans reduces plasma cholesterol by causing a redistribution of cholesterol between plasma and adipose tissues. The earlier studies by Frantz and Carey (5) showed a decrease in the liver cholesterol in patients given unsaturated fat diet. These studies indicate that unsaturated fats may increase the tissue cholesterol pool, probably in the adipose tissue (2) The failure of adipocytes to convert labelled glucose or acetate into cholesterol may imply that cholesterol in adipose tissue is derived from circulating lipoproteins or chylomicrons (2).

The significance of the plasma LCAT reaction is still not fully understood, but it is of major importance for the cholesterol homeostasis (9) It is known that it influences the amounts of unesterified cholesterol and lecithin in the erythrocytes (8 14). The lipid deposits in many other organs of patients with familial LCAT deficiency (6, 13) are also most probably consequences of the enzyme deficiency per se. The plasma LCAT reaction may therefore be of importance in the transport of unesterified cholesterol from peripheral tissues to the liver and reduced plasma LCAT activity may be associated with increased deposition of cholesterol in some tissues.

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A PILOT CLINICAL STUDY ON YERSINIOSES IN SOUTH EASTERN FINLAND

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Abstract. Yersinia agglutinins and—when suitable samples were available—also cultural and histological evidence of Yersinia infections have been searched for from 400 hospital patients during the period 1965–70 at Lappeenranta, Finland. In 31 of them immune responses compatible with infections caused by Yersinia pseudotuberculosis (YP) serotypes I, II, III or V or Yersinia enterocolitica (YE) serotypes 3 or 9 were found. In seven patients the diagnosis was confirmed by cultural or histological findings. YE serotype 9 was involved in 20 cases. Of the 31 patients 19 had disease states commonly regarded as immunological late effects of bacterial infections. Such states included erythema nodosum, temporary arthritides or arthralgias and also pulmonary changes resembling sarcoidosis. The joint symptoms met in children were indistinguishable from so-called benign aseptic arthritis. One patient had a typical Löfgren's syndrome.

Infections caused by other species of Yersinia genus than the plague bacillus have long been known among animals, especially in lagomorphs and rodents. During the last decade or so an increasing number of these infections, here called Yersinioses, have been discovered in man as well.

Before 1950 Yersinia pseudotuberculosis (YP) was known in human medicine only as a rare cause of septicaemia, but has since been shown to be the causative agent in many cases of mesenteric lymphadenitis and erythema nodosum and responsible for less clearly defined bowel and joint symptoms (2, 3, 11, 13, 16, 17, 24, 26, 27). Yersinia enterocolitica (YE) may also cause all these clinical syndromes, but has been especially incriminated in mild abdominal upsets best described as terminal ileitis (5, 30, 33, 38, 39).

Noteworthy among the clinical conditions more rarely connected with Yersinia infections are pneumonia, meningitis and unilateral Parinaud conjunctivitis (6, 9). In the Far East a local serotype of YP causes human infections characterized by scarlatiniform rashes (15).

The occurrence of Yersinia agglutinins and associated clinical syndromes is dealt with below on the basis of a series of patients accumulated during the diagnostic use of Yersinia serology in hospital practice in South-Eastern Finland.

MATERIAL

Since 1965 a Yersinia agglutination test using 7 YP and 2 YE strains (see below) has been performed in the District Public Health Laboratory of Lappeenranta, Finland. Serum samples for this test were sent by various clinicians mainly from patients suffering from indifferent abdominal pains, diarrhoea, erythema nodosum or joint symptoms.

Up to the end of 1970 one or more determinations of Yersinia agglutinins had been carried out in about 400 patients. Twenty-seven of them showed antibody responses to one of the seven Yersinia serotypes that do not cross-react with Salmonella group B or D antibodies (18, 21, 39). Twenty of these responses were against YE serotype 9, four against YE 3, two against YP I, and one against both YP III and YP V. From the great number of patients whose serum showed a raised and mostly permanent level of YP II agglutinins were included only those three cases in which lymph node biopsy revealed the characteristic histological picture of pseudotuberculosis. In addition to these, one of the YP I cases was verified histologically. In the other YP I case and in one case of YE 9 and YE 3 infection (Tables I and II) the corresponding Yersinia serotype was cultured from a lymph node removed at laparotomy. Other lymph nodes or stool specimens from the acute stage were not available for bacterial culture. Twenty-six of the cases were treated at the Central Hospital of Etelä-Saimaa. For the remaining five sufficient clinical data were obtained by inquiry. The period of observation varied from 1 month to 4 years.

Table I. Clinical picture. Yersinia agglutinins (titres expressed as reciprocals) and other relevant findings in child patients

Case no.	Clinical picture	Yersinia agglutinins: Serotype	Yersinia agglutinins: Highest titre	Significant changes in titre	Other findings
1	Mesenteric lymphadenitis	YE 9	12 800	Decreased to 800 in 3 mo.	Lymph node culture yielded YE 9
2	Mesenteric lymphadenitis	YE 3	3 200	Increased from 800 to 3 200 in 2 weeks	Lymph node culture yielded YE 3
3	Mesenteric lymphadenitis	YP I	3 200	Decreased to 100 in 3 mo.	Lymph node culture yielded YP I
4	Mesenteric lymphadenitis	YP II B	3 200		Characteristic histology in lymph node
5	Gastroenteritis febrilis	YE 3	1 600		
6	Gastroenteritis febrilis	YE 9	3 200	Decreased to 800 in 3 mo.	
7	Arthritis	YE 9	200	Decreased to 50 in 5 mo.	
8	Arthritis & erythema nodosum	YE 9	1 600		
9	Arthritis	YE 9	3 200	Decreased to 50 in 3 mo.	

CASE REPORTS

Case 15

A woman aged 42 had been appendectomized in 1966 for abdominal pain and found to suffer from mesenteric lymphadenitis with pseudotuberculous histology. Her serum had a raised permanent level of YP II B agglutinins, which during the following four years showed marginal fluctuations. During these years she had recurrent attacks of upper abdominal pain with diarrhoea lasting sometimes for several weeks. Many thorough attempts to demonstrate convincingly cholecystopathy, gastrointestinal ulcers, colon irritable or any other known pathological condition as the cause of her attacks failed repeatedly. When the subileus in 1970 was considered as a diagnostic possibility and was looked for in the radiological passage examination, the patient's attacks had subsided into different pains. No abnormal changes were revealed.

Case 24

At the age of 30 in 1960 this female patient got a febrile axillar lymphadenitis soon after her dog had died of an unknown disease associated with enlarged lymph nodes. The histological examination of the removed axillar node showed multiple neutrophile abscesses surrounded by epitheloid cells, i.e. a change fitting to pseudotuberculosis. At this time no clear cause for lymphadenitis was revealed. The blood picture and Paul-Bunnell test were normal. Antibodies to toxoplasma, cytomegalovirus and Francisella tularensis were not detected. The ESR was normal and the fever subsided quickly.

In the spring of 1967 she came again to hospital because of tender nodules in legs and joint pains after a febrile respiratory infection. Tuberculin test was strongly positive. In the serum was found a raised level of YP II B agglutinin which has since remained unchanged. No treatment was given and the symptoms subsided. In Jan. 1968 the nodules appeared again and this time the axillar nodes also became tender. As the histological changes discovered in the biopsy of leg nodules fitted to erythema nodosum, the patient was given this diagnosis. The fever was, however, lacking and the ESR normal. The nodules disappeared gradually only to recur after a minor respiratory illness in Dec. 1969. This time the patient also had a slight fever and her main complaint was pain and effusion in both knees. The lymph node biopsy from the neck again showed changes compatible with pseudotuberculous infection. The TB-bacilli were not at any time found from sputum or urine and the chest X-ray remained normal through the years. The examination of synovial fluid, serum haptoglobin level and clinical signs in joints suggested inflammatory rather than degenerative aetiology but no clues to any form of so-called collagen diseases were found. The arthritic symptoms passed away in four months.

Case 30

A woman aged 42 years developed pulmonary changes and elevated ESR (54 mm/h). As these were the only clinical signs, the diagnosis of sarcoidosis was seriously considered. Specimens obtained at bronchoscopy and mediastinoscopy however showed no histological changes attributable to sarcoidosis. The Mantoux with 2 tuberculin U/0.1 ml was negative and TB-bacilli could not be demonstrated in any specimen by staining or culturing. The tests for rheumatoid factors or FLH (farmer's lung hay) antibodies were also negative. All the pulmonary changes disappeared, however, in three months without any treatment.

Case 31

A 30-year-old woman developed typical erythema nodosum nodules in both legs after a febrile pharyngitis. At the same time she had migratory pains in the ankle, knee and hip joints. The left knee developed hydrops. The erythema nodosum changes and joint pains disappeared after a few weeks. The enlarged hilar lymph nodes, however, which were present during the whole of the erythema nodosum

Table II. *Clinical picture Yersinia agglutinins (titres expressed as reciprocals) and other relevant findings in adult patients*

Case no.	Clinical picture	Yersinia agglutinins: Serotype	Yersinia agglutinins: Highest titre	Significant changes in titre[a]	Other findings
10	Abdominal pains	YE 9	400		
11	Diarrhoea	YE 9	3 200		
12	Diarrhoea	YE 9	800		
13	Diarrhoea	YE 9	400	Decreased to 50 in 2 mo	
14	Mesenteric lymphadenitis	YP I A	800		Characteristic histology in lymph node
15	Mesenteric lymphadenitis	YP II B	1 600	Fluctuated between 200 and 1 600	Characteristic histology in lymph node
16	Unexplained low grade fever	YE 9	800		
17	Unexplained low grade fever	YE 9	1 600		
18	Prolonged fever	YE 9	800	Decreased to 50 in 10 mo.	
19	Septicaemia	YE 9	800	Remained at same level 16 mo.	Enlarged spleen and lymph nodes
20	Arthralgia	YE 9	800	Decreased to 100 in 6 mo	Febrile, enlarged lymph nodes
21	Arthralgia	YE 9	800	Decreased to 50 in 3 mo.	Febrile, bdominal pains
22	Arthralgia	YE 9	1 600	Decreased to 800 in 5 mo.	Previous diarrhoea
23	Arthralgia	YE 9	800		
24	Atypical erythema nodosum ith arthritis	YP II B	800	Remained at the same level during 8 mo	Characteristic histology in axillar and neck lymph nodes
25	Erythema nodosum	YE 9	3 200		
26	Erythema nodosum	YE 3	12 800	Decreased to 400 in 7 mo.	Swollen joints
27	Erythema nodosum	YE 9	800	Decreased to 100 in 4 mo	
28	Erythema nodosum	YE 9	3 200		S ollen joints
29	Erythema nodosum	YP III	200		
30	Sarcoidosis-like pulmonary changes	YE 3	1 600	Decreased to 400 in 10 mo.	No pathological findings in mediastino- or bronchoscopy
31	Pulmonary changes, arthralgia and erythema nodosum as in Löfgren's syndrome	YP V	400	Decreased to 25 in 12 mo.	No histological changes in lymph nodes obtained by scalenus biopsy

Twofold decreases in titres ere seen in cases 10, 11 23 25 and 28 which could be followed from 1 to 4 months.

period, lasted ten months. During the course of the disease the ESR rose to 38 mm/h. The Mantoux test with 10 tuberculin U/0.1 ml gave positive result. The scalenus biopsy revealed no significant changes. The immunoelec trophoresis and serum uric acid level were normal. The Waaler-Rose test was negative.

SEROLOGICAL METHODS

Except for the first 20 sera, hich were searched only for YP agglutinins, all sera were screened by slide agglutination against YP serotypes I A, I B, II A, II B, III, IV and V and YE serotypes 3 and 9. The YP strains were obtained from Dr Mair, England (23), YE 3 from Prof. Winblad, Sweden (5 38) and YE 9 from Dr Jansson, Finland (Nilehn strain no. 193) (29). The bacterial mass for agglutinations was taken directly from smooth colonies grown on plates incubated at 22°C. Each serum giving positive reaction in screening was titrated in tubes using live bacterial suspensions and twofold serum dilutions from 1 25 up to 1 12 800. The suspensions were prepared to 0.5% NaCl solution and their concentrations standardized by Coleman nephelocolorimeter. The tubes ere first kept for two hours at 37°C and then for two day at 4°C. The titres were read from bottom figures. The known positive and negative controls ere included in every titration and, if necessary the hole test was repeated until the interpretation as unequivocal.

The distinct agglutination (a smooth bacterial mat t the bottom of the tube) in the 1 200 or higher dilution was considered as evidence of raised agglutinin level. Atypically behaving, mostly permanent, raised titres (ad 1 1 600) often associated with lower titres to YP II A, YP III, YE 3 and YE 9 were found with YP II B suspensions. The absorption of these sera with the 0-antigens of Salmonella group B could not provide sound basis for their interpretation; and when an Enterobacter cloacae strain cultured from stool sample of an erythema nodosum patient was also agglutinated by these sera, it became evident that, besides the known sharing of an 0-antigen with Salmonella group B species, also other factors con-

tributed to the non-specificity and permanency of YP II B agglutinins. Except in the septicaemic patient (case 19), raised agglutinin titres in sera which did not react with YP II B were always associated with temporary antibody responses when the course of the response could be followed for at least three months. This kind of response to YE 3 developed in one patient (case 26) having permanent YP II B agglutinins.

RESULTS

Nine of the 31 specific agglutinin responses occurred in children under 15 years. Both sexes were equally represented and the age varied from 3 to 14 years. Some details of these cases are presented in Table I.

Four of the children were laparotomized because of symptoms simulating appendicitis, all of them had mesenteric lymphadenitis. The fifth child had also predominantly abdominal symptoms, but was clinically considered to be a case of gastroenteritis. Of the remaining four one presented as a case of unexplained prolonged fever and three as arthritis. One of the last three patients had also erythema nodosum. All arthritic cases had had fever and diarrhoea about two weeks before the joint symptoms. Their ESR were above 100 mm/h but the tests for rheumatoid factors and nuclear antibodies were negative. The antistreptolysin titres were not elevated and the chest X-ray pictures were normal. The arthritic symptoms consisted in all cases mainly of tenderness on movement and joint swelling, were polyarticular and at first migratory localizing gradually at specific joints. Two patients developed knee hydrops. The synovial fluid from the knees showed leucocyte counts up to 16 500/mm³ and low viscosity. There was no morning stiffness and no other radiological evidence of joint changes than the soft tissue swelling. The symptoms lasted in all cases about four months and recurred in one of them. None however retained any permanent symptoms.

Of the 22 adult cases 7 were men and 15 women. The ages varied from 16 to 65 years mean 39. According to the prominent clinical features the adult cases can be grouped in five different categories: the abdominal, febrile, arthralgic, erythema nodosum and pulmonary cases (Table II).

1. Of six abdominal cases two had surgically proven mesenteric lymphadenitis, others indifferent abdominal pain and diarrhoea. One of the adenitis cases (no. 15) developed after the laparotomy into a diagnostic problem which could not be definitely solved. We have described this case above because the recurrence of subileus, which may be caused by Yersinia (18) could have been one explanation of her later symptoms.

2. One of the four febrile cases (no. 19) had a definitely septicaemic disease with enlarged spleen and lymph nodes. The lymph node biopsy revealed no specific changes. He received a prolonged course of streptomycin (20 g), during which he recovered gradually. In one of the other patients, who had a squirrel and a parrot as pets at home, the fever lasted several months. In both these cases the antibiotic treatment was unfortunately begun without previous blood culture. In all patients the agglutinin response to Yersinia strains was the only clue to the aetiology of the disease although a thorough microbiological and clinical investigation was made to discover other causes of unexplained fevers. The recovery was complete in all cases.

3. Diarrhoea preceded the symptoms in two of the four joint patients, respiratory infection in the remaining two. The joint symptoms were of arthralgic type consisting of pain and tenderness on movement. No morning stiffness or radiological changes were seen. In all patients except one in whom only two joints were permanently affected, the symptoms involved many joints, asymmetrically and in varying numbers. The ESR was slightly elevated (up to 30) only in two patients. The tests for rheumatoid factors and nuclear antibodies were negative in all. The antistreptolysin titres were within normal limits. The symptoms lasted from two to four months no recurrences were seen.

4. Five of the six patients in this group had the classical erythema nodosum nodules in lower extremities, with prolonged fever which subsided after some weeks as the painful nodules disappeared. Diarrhoea preceded the symptoms in three cases. Antistreptolysin titres were not elevated in any case and Latex and Waaler Rose tests were negative. In one exceptional case the tender subcutaneous nodules lacked the usual discoloration and were not accompanied by fever. The course of the disease in this patient (no 24), who had also axillar and neck lymphadenitis and arthritis, has been described above.

5. One of the pulmonary cases (no. 31) had



Fig. 1. The polycyclic enlargement of hilar glands in case 11 (Löfgren's syndrome).

polycyclic enlargement of the hilar lymph nodes (Fig. 1) in the other (no. 30) the pulmonary changes comprised prominent hilar regions and patchy infiltrations in both lungs, i.e. radiogram which could well have been classified as a second degree sarcoidosis.

DISCUSSION

The evidence that all of our cases were really Yersinioses is of course not watertight, because in 24 instances it was based on specific agglutinin responses only. The following facts, however, suggest this possibility: 1. In none of them was any other cause of the disease found. The kinds of responses accepted as evidence for Yersiniosis are not known to occur in other infections. Brucellosis and cat scratch fever are so far unknown in Finland. 3. In every case in which satisfactory specimen for cultural or histological examination was obtained, the result of this examination confirmed the serological diagnosis. 4. The majority of the disease states occurring in our patients were those previously described in Yersinia infections. 5. The relative frequency of various serotypes in our series is about the same as in culturally documented Finnish cases (1).

Of course future studies may show more lapses in the specificity of Yersinia aggl[illegible] verify that pulmonary changes [illegible] toms described above in cases [illegible] really be caused by Yersinia [illegible] range of the clinical condit[illegible] Yersinioses can only be reve[illegible] the patients with reasonable [illegible] fections.

Our cases establish, how[illegible] doubt that Yersiniosis is [illegible] South-Eastern Finland [illegible] mainly due to the freq[illegible] fections (37). Instead of [illegible] Sweden is the most [illegible] monest serotype of YE [illegible] Eastern as well as [illegible] This YE serotype h[illegible] occasionally in Swe[illegible] collected many YE [illegible] America, found [illegible] 9 also from Belg[illegible] ond commonest [illegible] YE 3. The YP [illegible] Yersinia infec[illegible] Great Britain, [illegible] had no prom[illegible] son for this [illegible]

our YP patients, but in this connection it is interesting to note that the commonest YP serotypes in Japan have so far been IV and V (36). Taken together with these and other observations of the incidence of various Yersinia serotypes in the cases of Yersiniosis reported from different countries, our findings suggest considerable geographical variations in Yersinia types causing the human infections. Such variations may reflect the differences in animal Yersinioses prevalent in various geographical regions or also different modes of transmission of these agents.

The clinical picture in our arthritis cases had points in common with rheumatic fever (7) and juvenile rheumatoid arthritis, but also and especially with so-called benign aseptic arthritis, which seems nowadays to be the commonest joint disorder among children (10, 12) The aetiology of benign aseptic arthritis is still unsolved. It should be evidently included in the group of reactive infectious arthrites. Our results seem to indicate that many of these cases are due to Yersinia. Ahvonen has found a high percentage of Yersinia agglutinins in his large material of arthritis in children (1).

There is still much disagreement about the diagnostic criteria for juvenile rheumatoid arthritis. In an extensive series of patients the recovery took place during the first year in 5 % of cases (19). In view of the facts that tests for rheumatoid factors remain mostly negative and X-ray changes sometimes develop very late (3) it is probable that cases caused by Yersinia infections have previously been diagnosed as juvenile rheumatoid arthritis

The localization of primary Yersinia infections in the ileocoecal region has caused some speculation about the relationships between Yersinioses and Crohn's disease (18, 25). No connection between them has, however been established. The negative outcome of Yersinia examinations in one case of Crohn's disease treated in our hospital accords with this finding The search for Yersinia aetiology only in cases where the symptoms fit mesenteric lymphadenitis or terminal ileitis may however restrict our views about the abdominal Yersinioses. Case 15 illustrates this possibility The consideration of Yersinia aetiology in other abdominal cases (31) or recurrent bouts of diarrhoea could establish whether Yersinia bacilli can infect the intestines chronically

Because of the resemblance of pseudotuberculous histology to that caused by Francisella tularensis and by the infective agent of cat scratch fever some experts consider the reticular abscess-forming inflammation evidence of YP infections only when met in mesenteric lymph nodes (17 25). Our case 14 where this type of histological change occurred in axillar and neck lymph nodes, together with the increasing level of YP II B agglutinins, could have provided clear evidence of Yersinia infections of other lymph nodes, too, if only the agglutinins had not been against the serotype known to give many unspecific reactions. The actual significance of the permanently increased levels of YP II B agglutinins, which we found in up to 10 % of studied sera, constitutes a manifold problem and a nuisance in Yersinia serology Without positive cultures they cannot, however be regarded as evidence of the common occurrence of YP II B infections in South Eastern Finland, although this possibility cannot be excluded.

The inflammatory response of experimental animals to intratracheal inoculation of YP comprises initial confluent pneumonic infiltrates and the gradual development after 4–5 days of abscess-forming reticular changes in peribronchial and peritracheal lymph nodes (25). Although the ability of YP or YE to cause similar pulmonary changes in man is not clearly established, Girard et al. (8) have described polycyclical enlargement of hilar lymph nodes in one of their mesenteric lymphadenitis cases with proven YP aetiology

On the other hand Winblad (39) using boiled YE antigens, did not find increased agglutinin titres in sarcoidosis patients with erythema nodosum. Clinical findings in our two pulmonary patients (see above, group 5) were indistinguishable from those of sarcoidosis. Besides the chest X-ray compatible with second degree sarcoidosis in case 30 the triad of erythema nodosum, perihilar lymph node enlargement and joint symptoms in case 31 fits well to primary sarcoidosis as described by Löfgren (20) Thorough attempts to demonstrate in biopsy specimens the typical histology of sarcoidosis failed, however in both cases. The Kveim test was not performed. On the basis of these observations we are not suggesting that sarcoidosis can be caused by Yersinia bacilli, but that some clinical symptoms, which are commonly believed to be typical of sarcoidosis (34, 35), may also be found in Yersinia infections. The

frequent occurrence of temporary clinical conditions simulating sarcoidosis, in which this or another aetiology can not be established, has already been documented in the scientific literature (4).

Even if one considers the unrepresentative selection of the cases, they still include an unexpectedly large number of patients having clinical syndromes such as erythema nodosum or aseptic arthritis, which are commonly regarded as immunological late effects of bacterial infections. Indeed, the considerable variety of protracted clinical conditions displayed by our patients seems to indicate that in Yersinioses the symptoms due to altered host response are often more prominent than the inflammatory effects caused by bacterial infection itself. *The joint symptoms peculiar to aseptic benign* arthritis considerably resemble those found in serum disease (14). Antigenic stimulation of reticular tissue and associated effects caused by antibody formation have been suggested as the mechanism producing abscess-forming reticular lymphadenitis in YP infections (25). In contrast to the two or three days needed for the full development of delayed skin recations, the skin test with YP antigen gives the most intense reaction on the first day following the injection (40).

To us all of these points suggest that the Arthus type immunological reaction plays an important part in the symptoms of Yersinioses. The application of the methods of experimental immunology to the pathogenesis of Yersinia infections might prove fruitful in the study of immunologic inflammatory mechanisms provoked by bacteria. From the clinical point of view however it would be useful to know how great a proportion of the cases of infectious arthritis, so often causing diagnostic difficulties, do in fact develop on the basis of Yersinia infection. For this to be possible, it is essential that clinical criteria and bacteriological and serological tests are carefully and simultaneously applied to a representative series of patients.

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ELECTROCARDIOGRAPHIC CHANGES DURING SELECTIVE CORONARY ANGIOGRAPHY

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Abstract During selective coronary angiography ECG was recorded in leads I and II in 95 consecutive patients with the purpose of studying the ECG changes invoked by the injection of 7 ml Urografin 60% (Schering). Marked but transient changes were seen, probably caused by the physico chemical properties of the contrast medium. The selective injection of the same volume of Ringer solution did not evoke any ECG changes. When blood volume in the coronary circulation is replaced by contrast medium or Ringer solution, transient coronary hypoxia will result. This hypoxia, therefore, is not the cause of the ECG changes observed in coronary angiography. Selective coronary angiography appears to impair depolarization as well as repolarization, the latter being the most affected function. Absence of ECG response during coronary injections indicates severe coronary artery disease or the presence of single coronary artery. Right coronary injection is followed by an increase in PQ interval. Arrhythmias seldom occur but are more likely to appear during right than during left coronary injections. The changes observed in this series are increased QRS and QT duration, shift in QRS axis in the frontal plane, which is clockwise during right and counter-clockwise during left coronary injections. The QRS vector is doubled. The T axis rotates in the frontal plane towards +120° during left and towards −75 during right coronary injection. When the T axis has this direction before angiography no shift in axis is seen during injection of contrast medium.

During left heart angiography small and transient ECG changes are usually seen (2). During selective coronary angiography however marked changes in the QRS and T waves are generally observed. The ECG follows a characteristic pattern. The mean QRS vector shifts to the left during left, and to the right during right coronary angiography while the mean T vector shifts in the opposite direction (2, 3, 7).

In patients with complete coronary artery occlusions small ECG changes are observed (7). In cases with prominent collateral flow a dual change in the ECG pattern may be seen during injection in the open artery. The expected ECG changes appear but are shortly afterwards followed by changes in the opposite direction, which are seen when the contrast medium reaches the myocardium supplied by the collaterals (2).

In the present investigation we have correlated the ECG response during selective coronary angiography to the different patient groups examined.

MATERIAL AND METHODS

Ninety-seven examinations have been carried out in 95 patients. In 19 patients only left and in 3 only right coronary angiography was performed. T   patients, both with old myocardial infarction, were examined twice. Twenty-six patients had aortic valvular disease, 40 coronary heart disease, 9 diffuse cardiomyopathy, 2 single coronary arteries, and in 18 patients examined because of anginoidal pains no obvious organic heart disease was found.

All examinations have been performed according to the Judkins technique (6) with the modification that cineangiography was used exclusively and with the injection of 7 ml Urografin 60% (Schering) by hand pressure. Usually 3 injections were introduced into the left and into the right coronary artery.

Standard ECG leads I and II were recorded on an Elema mingograph with paper speed 50 mm sec. The ECG was recorded continuously during the selective angiography in either coronary artery and the analysis was referred to that part of the ECG recording in which the most pronounced changes appeared. Vectors were calculated in Ashman units based on counting of the small squares under the QRS and T waves respectively. Amplitudes were read to the nearest 0.5 mm and time intervals to the nearest 0.01 sec.

I   5 patients Ringer solution was injected selectively into the coronary arteries in the same manner as the contrast medium, and the ECG was recorded as described above.

Table I *Mean QRS axis and vector during selective coronary angiography in different patient groups (vectors in Ashman units)*

		Before angiography		During left selective angiography		During right selective angiography	
Patient group	No. of pats.	Mean axis	Mean vector	Mean axis	Mean vector	Mean axis	Mean vector
No organic heart disease	18	+33	5.9	−8	11.6	+87°	14.0
Cardiomyopathy	9	+7°	7.1	−29°	10.0	+59°	8.0
Aortic valvular lesion without coronary disease	15	+26	8.4	+12°	10.7	+57	13.4
Aortic valvular lesion with coronary disease	11	+23°	9.9	−2°	13.5	+57°	14.0
Angina pectoris	13	+29°	5.2	+50°	7.6	+54	7.6
Old myocardial infarction	15	+7°	4.3	−30°	7.2	+38°	7.0
Ventricular aneurysm	14	+1	4.6	−14°	6.7	+34	6.7
Single left coronary artery	1	−5°	4.0	+18°	5.0		
Single right coronary artery	1	−45°	29.0			−52°	28.0
Mean all cases	97	+11	6.5	−5°	9.6	+56	10.8

RESULTS

The ECG changes were most marked in the QRS and T waves. They appeared after 2–3 sec with a maximum of about 5–6 sec after the start of the injection. In many cases the maximum change in QRS appeared about 1 sec after the maximum change in the T wave. After another few seconds the ECG gradually normalized, the most prominent changes being visible in a period of 10–15 sec after the injection. Usually complete normalization did not occur until 1 min had passed. In the frontal plane the mean QRS axis usually moved left during left and right during right injections (Table I). The mean T axis rotated in the opposite direction (Table II). Considerable individual variations were observed. The mean T axis seemed in cases with no organic heart disease to go towards an average of about + 120 during left and about − 60 during right angiography. The shift in the mean axis was greater the more the direction before angiography differed from these values (Figs. 1 and 2). To a certain degree

Table II *Mean T axis and vector during selective coronary angiography in different patient groups (vectors in Ashman units)*

		Before angiography		During left selective angiography		During right selective angiography	
Patient group	N of pats.	Mean axis	Mean vector	Mean axis	Mean vector	Mean axis	Mean vector
No organic heart disease	18	+63°	5.6	+121	40.1	−55°	44.7
Cardiomyopathy	9	+75°	3.4	+109°	19.1	−54	22.8
Aortic valvular lesion without coronary disease	15	+143°	6.2	+141	24.0	−68°	39.3
Aortic valvular lesion with coronary disease	11	+164°	6.0	+136°	23.9	−82°	39.1
Angina pectoris	13	+47°	4.2	+86°	17.4	−18°	16.3
Old myocardial infarction	15	+69°	2.5	+85°	13.9	−28°	22.4
Ventricular aneurysm	14	+57°	3.6	+97°	10.6	+19°	9.8
Single left coronary artery	1	+48°	6.0	+42°	6.0		
Single right coronary artery	1	+84°	17.0			+69°	39.0
Mean all cases	97	+86°	4.8	+111	22.0	−37°	28.8

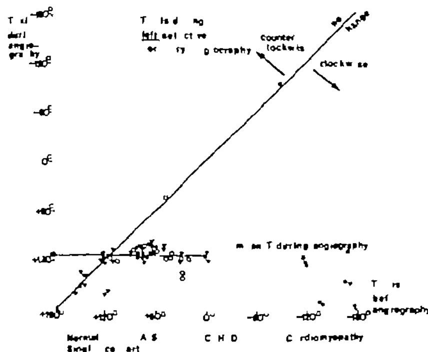


Fig. 1 The T axis during left selective coronary angiography related to the T axis before angiography The T axis seems to deviate towards direction of about +120° irrespective of the direction befor angiography Exceptions are seen in patients with coronary heart disease. Normal = no organic heart disease, A.S. = alvular aortic stenosis, C.H D. = coronary heart disease.

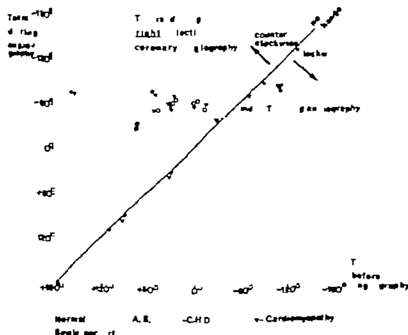


Fig. 2 The T axis during right selective coronary angiography related to the T xis before angiography The T axis seems to deviate towards direction af obut −60° when patients ith coronary heart disease are excluded. Mean T axis deviation for all patients ( ) is −37°

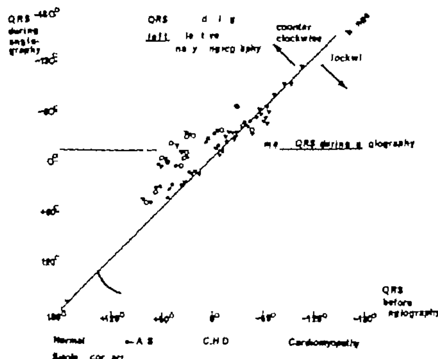


Fig 3 The QRS axis during left selective coronary angiography related to the QRS axis before angiography Marked individual variations exist, but in most patients the QRS axis rotates counter-clockwise.

the mean QRS axis also seemed to rotate towards a fixed direction during selective angiography (Figs. 3 and 4).

The QRS vector was on an average doubled in patients with no organic heart disease (Table I) and the T vector was increased 8 times (Table II) compared with the vectors before the contrast injection

In the 18 patients who had no organic heart disease the ECG response during selective coronary angiography followed a rather constant pattern. The T axis deviation was during left angiography between +101 and +148 the mean being +121 During right angiography the deviation was between −35 and −78 with a mean deviation of −57 In the QRS axis a somewhat greater variation was seen.

In 26 patients with aortic stenosis a greater variation in QRS and T axis was observed The T axis was directed more towards the right both before and during angiography The mean deviation was +139 during left and −74 during right angiography Eleven of these patients had coronary atheromatosis and 7 of them also occlusions in main branches of the left coronary artery The ECG changes observed during angiography did not differ significantly in these patients from those observed in the other patients with aortic stenosis.

Nine patients had cardiomyopathy Coronary disease was not observed. The ECG had low voltage and during angiography only small changes in the QRS complex were observed. The T wave changes were more marked and followed the usual pattern.

Two patients had single coronary arteries. In both only small ECG changes were observed during selective coronary angiography

Altogether 4 injections were done in 40 patients with coronary heart disease. Coronary artery occlusions were seen in 35 patients, in 7 only extensive atheromatosis was visualized. In these patients the amplitude of the QRS as well as the T vectors was usually lower during selective coronary angiography than in patients with no coronary disease. Fourteen patients had left ventricular aneurysm after myocardial infarction and the ECG responses to selective angiography were small or absent.

The magnitude of ECG changes seen during selective coronary angiography seems to be related to the presence of occlusive coronary arterial dis-

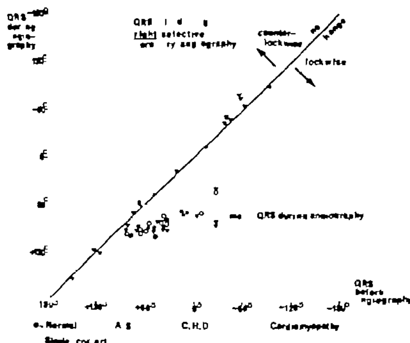


Fig. 4. The QRS axis during right selective coronary angiography related to the QRS axis before angiography. Marked individual variations exist, but in most patients the QRS axis rotates clockwise.

case (Tables III and IV). A marked deviation from the normal was the rule when occlusions were diagnosed in either the right or both branches of the left coronary artery. In patients with occlusions in either the anterior descending or the circumflex artery only a minor deviation from the normal response was seen. Nine out of ten patients with occlusive coronary disease had angiographic signs of collateral circulation. A dual shift in ECG pattern has been reported by others (2), but was observed only in three patients in this study.

An increased QRS duration during angiography was observed in 41 cases. In 19 this was noted both during left and right coronary injections. In 13 patients the increased QRS duration was only observed during left and in 9 only during right injections. In 7 cases the QRS complex was prolonged by 0.03 sec or more. In the remaining 36 cases only a small increase was observed (0.01–

Table III. *QRS axis and vector during selective coronary angiography in 33 patients with occlusive coronary disease (vectors in Ashman units)*

Occluded coronary artery	No. of pats.	Before angiography		During left selective angiography		During right selective angiography	
		Mean axis	Mean vector	Mean axis	Mean vector	Mean axis	Mean vector
Right	6	+40°	4.7	+2°	4.2	+76°	5.0
Right and circumflex	4	+1	2.8	+2°	5.3	+6°	3.0
Anterior descending and circumflex	3	−28	9.0	+19°	13.3	−9°	12.3
Anterior descending	14	−1	4.1	+2°	7.3	+46°	8.5
Circumflex	6	+4	6.7	−21	10.7	+66°	9.0

Table IV *Mean T axis and vector during selective coronary angiography in 33 patients with occlusive coronary disease (vectors in Ashman units)*

Occluded coronary artery	No. of pats.	Before angiography		During left selective angiography		During right selective angiography	
		Mean axis	Mean vector	Mean axis	Mean vector	Mean axis	Mean vector
Right	6	+5°	4.2	+27°	7.5	−4	6.3
Right and circumflex	4	−66°	1.3	+4	8.8	+27°	3 0
Anterior descending and circumflex	3	+64	8.3	+107°	21.0	+14	27.3
Anterior descending	14	+98°	3.3	+116°	12.6	−16°	20 1
Circumflex	6	+44	3 0	+123	24.5	−70°	31.6

0 0_ sec) A significant prolongation of the QT interval was seen in 60 cases during left and in 44 during right coronary injections. The prolongation was due to a greater duration of the T wave per se.

The ST changes were usually small (0.5–2 mm) and were seen in all patients during selective angiography either in a single or in several injections. The ST interval deviated in the same direction as the deviation in the T wave. During left coronary injection a depression of the ST interval was seen in lead I and an elevation in lead II The opposite was recorded when the injection was done in the right coronary artery More extensive changes were seen (2 5–3 mm) in 9 cases.

Changes in the P wave were difficult to evaluate from the registrations used in this study In lead II a slight depression in amplitude (0.5–1 mm) was seen in 7 cases during left and in 14 cases during right injections.

The heart rate was usually retarded during selective coronary angiography Mean heart rate before injection was 80 8. Injection of contrast medium retarded the heart rate to 71 6 during left and 63 7 during right injections A rise in heart rate was seen in two cases during left and in another during left as well as right injection. Thirteen patients had no change in heart rate during left angiography

An increase in PQ interval was seen in 22 patients, all during right angiography Two had asystole of 3 and 16 sec duration respectively and two had AV block with Wenckebach's periods and one AV block of first degree. The AV blocks disappeared after a few seconds. In 17 other cases only a moderate prolongation of the PQ time (0.01–0.02 sec) was seen.

Two patients developed ventricular fibrillation during right coronary angiography and were successfully defibrillated. Seven patients had a few ventricular ectopic beats and one a transient coronary sinus rhythm during injection in the right coronary artery During injection in the left coronary artery no tachyarrhythmia was observed.

In order to see whether the ECG response due to selective coronary angiography was caused by hypoxia induced by the volume of contrast medium injected, 7 ml of Ringer solution was injected selectively in the coronary arteries in the same manner as the contrast medium in three patients. No ECG changes were observed during this procedure, although all patients had marked QRS and T changes during selective coronary angiography (Fig. 5).

COMMENTS

During selective coronary angiography marked but transient changes in ECG usually occur The ECG response is strictly a gradual change and the most distinct alteration is usually seen between 4 and 6 sec after the start of contrast injection. After a few seconds the ECG gradually reverts to the initial pattern, which is reached during the next 30 to 60 sec. This sequence indicates that the ECG change is due to a process which develops and abates gradually The most probable location for such a process is at a peripheral level within the myocardial cells or in the most peripheral part of the conductive tissues.

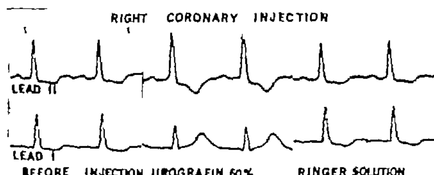


Fig 5 ECG recording during right selective coronary angiography and during selective injection of equal volume of Ringer solution in the right coronary artery

ECG changes did not develop during the injection of Ringer solution. This indicates that hypoxia cannot be the cause of the ECG response when blood volume is displaced by contrast medium. It has been reported that the side-effects of contrast media injected in angiocardiography are proportional to the concentration of sodium (1 5). Another possibility is the high viscosity of the contrast medium, which may impede the microcirculation.

As previously demonstrated, distinctive changes are usually seen in the QRS and T waves (2, 3 7). During injection in the left coronary artery the QRS vector rotates counter-clockwise and the T vector clockwise. This has been described as a "left coronary response (2). Right coronary injections are followed by a right coronary response and the vectors rotate in the opposite direction. The QRS complex corresponds to a left anterior hemiblock during left coronary injection and to a left posterior hemiblock during right coronary injection (4 8).

A more detailed analysis of the present material seems to indicate that the T axis in the frontal plane rotates in the direction of +120 during left and −60 during right coronary injections. The more the initial T axis departs from these values, the greater T axis deviation should be expected after angiography. Similarly the more the initial T axis equals these values, the more insignificant will the post-angiographic T axis deviation be. In patients with aortic stenosis the T axis is directed even more rightwards. During left coronary injection the T axis deviation may approach +140 and during right coronary injection −75 This is probably due to left ventricular hypertrophy

In this study the QRS duration remained unchanged or increased, the vectors usually increased and the mean axis rotated towards the area supplied by the injected artery These changes indicate a delay in depolarization in the area invaded by contrast medium. The T wave changes consist of an increased QT duration distinct increase in vectors and a rotation of the mean T axis away from the area supplied by the injected artery This area, therefore, appears to be electrically negatively related to the other parts of the left ventricle Thus repolarization seems to be delayed in the area invaded by contrast medium. Selective coronary angiography therefore, seems temporarily to affect the myocardial function, with impairment of both depolarization and repolarization. The T wave changes ar more marked than the QRS changes and individual variations are less pronounced. This may indicate that repolarization is the most affected function.

Although the ECG changes during selective coronary angiography are marked, complications due to the procedure are relatively infrequent. An explanation in this respect may be the very rapid regression of the ECG changes.

Minor to moderate coronary artery disease, including occlusions in one of the left coronary branches, does not seem to alter significantly the ECG pattern already described during selective coronary angiography In patients with occlusions of major branches of the coronary circulation, however the ECG changes described previously are not seen As reported by Coskey and gid

son (2) and MacAlpin et al. (7) only small ECG changes are observed during selective coronary angiography both during injection in the occluded artery and in the opposite one.

In two patients with single coronary arteries and adequate blood flow no ECG changes were seen. Thus the absence of ECG changes during selective coronary angiography indicates major coronary artery pathology or the presence of a single coronary artery

In patients with coronary artery occlusions and collateral circulation from the opposite coronary artery a dual shift in the ECG response may be seen during selective coronary angiography Firstly one observes the pattern anticipated with regard to the artery injected. Secondly a change of pattern occurs as if the injection had been made in the contralateral artery (2, 7) In the present study this dual shift was only seen in three patients.

Serious rhythm disturbances are infrequent and are mainly seen during right coronary injections. The two episodes of ventricular fibrillation in this series occurred during a right coronary angiography

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PERIARTERITIS NODOSA

A Ten-Year Follow-up Study of Ten Cases

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Abstract. Ten cases of periarteritis nodosa have been histologically diagnosed during a 3-year period, nine of them by biopsies. Most of the patients had gastrointestinal and cardiac symptoms, together with fever. The BSR was high in nine cases, and in all ten patients serum electrophoresis revealed hypalbuminaemia. The γ- and α_2-globulin levels were increased in many cases. Six patients were anaemic. Proteinuria was present in six cases. All the patients were treated with corticosteroids. Seven patients were alive after 5 years, and five after 10 years.

Periarteritis nodosa (PN) was first described by Kussmaul and Maier (4) in 1866. It is still a rare disease, but during recent decades many more cases have been reported than during the 67 years following the primary report (9). The apparent increase in frequency finds an explanation in improved medical facilities and in the more common histological examination of specimens obtained on biopsy and autopsy.

The vascular process in PN may affect any organ (2). The kidneys are involved in about 80% of the cases, the heart in 70, the liver in 60, the mesenterium in 30, the muscles in 30, the pancreas in 25, the skin also in 25, and the central nervous system in 8% (12). Naturally the localization of the process influences the quality of the symptoms and the prognosis. Rose and Spencer (8) found that cases with lung lesions were particularly distinct from cases with no involvement of the lungs. In their series the lungs were involved in 32 cases out of 104. The commonest clinical symptoms are fever, general weakness, peripheral neuritis, intermittent haematuria, abdominal and articular pain, myalgia, hypertension and tachycardia. Thus the clinical signs of PN are variable, and as a rule histological criteria are required for the final diagnosis.

In most cases of PN the diagnosis has been made post mortem (11) with the prognosis consequently being considered as extremely poor. Follow-up studies are more reliable for prognosis. In most reports of this kind the patients have been followed up for 5 years after establishment of the diagnosis by biopsy. As PN was diagnosed in 10 cases during a 3-year period at a regional hospital, in 9 of them intra vitam, a report on this group of patients may be of general interest now, more than 10 years later.

MATERIAL

During a 3-year period (July 1956 to June 1959) 10 patients admitted to the Central Hospital of Vasa were found to suffer from PN. Their ages varied from 43 to 64 years. In 9 cases the diagnostic histological specimen was obtained by biopsy and in one case by autopsy. Some of the histological examinations were made at the Institute of Pathology of the University of Helsinki, and others at the Institute of Pathology of the University of Turku.

RESULTS

Clinical symptoms

The main symptoms are listed in Table I. Most patients had abdominal complaints and fever, and in more than half of the patients cardiac symptoms were recorded. Skin changes were noted in one half of them, and half had diffuse or articular pains in the limbs.

The gastrointestinal symptoms, present in 9 of the 10 patients, varied from case to case. Most of them had diffuse abdominal pain, which led 1

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Table I. *Symptoms and survival in 10 cases of PN*

Mult. = multiple organs studied at autopsy
Symptoms present are indicated by +

Case no. ...	1	2	3	4	5	6	7	8	9	10	
Sex	♀	♀	♂	♀	♂	♀	♀	♀	♂	♂	
Age (y) ...	58	52	45	43	50	64	50	59	48	53	
PAD from	Skin	Liver	Mult.	Muscle	Muscle	Muscle	Muscle	Appendix	Liver	Liver	Total
Gastrointestinal symptoms		−	+	+	+	+	+	+	+	+	9
Fever	+	−	+	+		+	+	+		+	8
Cardiac symptoms			+	+	+	+	+			+	6
Cutaneous symptoms	+					+	+	+	+		5
Articular pain						+		+	+		3
Diffuse pain	+		+					+	+		4
Hypertension		+		+		+					3
Ophthalmic symptoms		+		+					+		3
Cerebral symptoms				+	+		+				3
Pulmonary symptoms			+				+				2
BSR > 20 mm/h	+	+	+	+		+	+	+	+	+	9
Hb 10 g/100 ml	+	+		−		+			+	+	6
Leukocytes > 10 000	+		+	+	+				+		5
Eosinophils > 5 %	+			+						+	3
NPN > 50 mg/100 ml			+	+		+					3
Proteinuria	+		−	+		+		+		+	6
Haematuria						+					1
Alive after 10 years	Yes	Yes	No	No	Yes	N	N	Yes	Yes	No	5
Survival time after diagnosis (y)			< 1	2		6	1			8	

performance of a large number of X-ray examinations. In 2 cases a pathological gall bladder was found, and in one case signs of atypical colitis. In one case an exploratory laparotomy was performed. Macroscopically nothing of interest was found, but the appendix was removed. In this case histological signs of PN were found in the appendix and the omentum.

In 8 cases fever of long duration was encountered, usually irregularly intermittent, and rather mild.

Four patients had tachycardia. In 2 cases distinctly abnormal ECGs were obtained, indicating myocardial infarction in one patient and diffuse myocarditis in the other.

In 2 cases the skin of the legs was of a coldish-blue shade. One patient had skin changes of the livedo reticularis type, and had had erythema nodosum for several years.

Four patients had diffuse pain in the limbs. Three of them, and one additional patient, had tender leg joints. One of them seemed to have typical rheumatoid arthritis, with swollen joints and roentgenological changes.

The eye symptoms encountered in three cases were keratoconjunctivitis sicca in one patient, iritis in one and hypertensive retinal changes in the third.

Two patients were suffering from the sequelae of apoplectic seizures when first admitted to the hospital, and one patient had a stroke during the initial period of observation. In addition, one patient (no. 2) had a cerebral apoplexy 4 years later. Marked nervousness and lability were noted in three patients.

Transient pulmonary infiltrates, resembling those in the Löffler syndrome, but without eosinophilia, were noted in one case. Another patient had haemoptysis, but the chest X ray film was normal. In one case, the chest X ray revealed infiltrates, indicating old tuberculous changes.

Laboratory findings

Most patients had an elevated BSR (Table I). In 6 cases the values recorded exceeded 100 mm h. A slight eosinophilia in the peripheral blood was noted in 2 cases and marked eosinophilia (above 40 %) in one case. These patients did not exhibit any pulmonary changes. Six of the patients were anaemic. Proteinuria was found in 6 patients. Three of them had elevated serum non-protein nitrogen and one of them transient microscopical

Table II. *Results of serum filter paper electrophoresis in 10 patients with PN*

Normal values within parentheses (6)

Case no.	Total (6.4–8.1)	Albumin (3.5–5.2)	α_1 (0.2–0.4)	α_2 (0.4–0.8)	β (0.6–1.0)	γ (0.7–1.6)
1	5.9	2.1	0.3	0.6	0.9	1.9
2	7.1	2.5	0.1	0.6	0.8	3.1
3	6.5	3.1	0.2	0.8	1.0	1.4
4	6.6	2.8	0.1	0.6	1.1	2.0
5	7.4	2.3	0.2	1.2	1.2	2.6
6	6.3	2.8	0.3	0.9	1.0	1.3
7	8.3	3.4	0.3	1.0	1.4	2.2
8	6.4	2.3	0.3	0.6	0.6	2.5
9	6.3	2.2	0.6	1.1	1.0	1.4
10	7.8	2.5	0.5	1.1	0.9	2.8

haematuria. The latex test was positive in one case, with no clinical signs of rheumatoid arthritis. A false positive Kahn test was found once (no. 1) and serum cryoglobulin in one case (no. 10). The LE-cell test was negative in all cases, but in one patient (no. 8) a positive immunofluorescence reaction for nuclear antibodies was noted during the follow-up period. This patient had transient thrombocytopenia some years after the initial study. The results of serum filter paper electrophoresis are listed in Table II. Hypalbuminaemia was a consistent finding. The γ-globulin fraction was elevated in 7 cases, and the α_2-fraction in 5.

Treatment and survival

All the patients were treated with corticosteroids. The drugs were administered for short periods to patient no. 3 who died in the hospital, and to nos. 5 and 9. All the other patients were treated with corticosteroids for several years.

One patient died in the hospital. In this case an autopsy was performed and the diagnosis was established post mortem by means of histological specimens from several organs. Four patients died at home and autopsies were not performed. The probable causes of death were malignant hypertension with uraemia (no. 4), haematemesis (no. 6), cerebral haemorrhagy (no. 7) and myocardial infarction (no. 10). Two of these patients lived more than 5 years after the diagnosis. Five patients were still alive after 10 years.

DISCUSSION

Periarteritis nodosa may be suspected on clinical grounds, but a positive histological result is required for definite diagnosis. In this group of 10 patients the diagnosis was established intra vitam in 9 cases by means of biopsies. Biopsy specimens are conveniently obtained from the skin or muscles, but blind biopsies from unaffected organs are rarely helpful (2, 5). If possible, the biopsy should be taken from an organ presenting clinical symptoms. Nevertheless, in our group of patients, typical arteritic changes were found in biopsies from the gastrocnemius muscle in 4 cases, even if the patients had no pain in this region. In 3 cases the diagnosis was established by means of needle biopsies from the liver. All of these patients had abdominal symptoms, but the liver function tests were normal, or almost normal, in all of them. Non-specific arteritic changes may be found in the appendix (7). In our patient with arteritis of the appendix, PN-type changes were also observed in the omentum. The only positive skin biopsy in our series was obtained from a region displaying reddish-brown infiltrates. Kidney biopsy may contribute to the diagnosis in many cases, but during the 1950' this was not a routine procedure in most Finnish hospitals.

The prognosis of PN is grave. Formerly the diagnosis was usually made at autopsy and the course was considered uniformly lethal. In most retrospective autopsy studies the time from the onset of symptoms to death has been less than one year (10). As more and more cases have been diagnosed by means of biopsies from living subjects, milder cases have been recorded and the prognosis has improved (2). In Bleckman's series (1) the diagnosis was established at autopsy in 12 cases and by biopsy in 2. Both the last me

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Ophthalmic symptoms		+		+					+		3
Cerebral symptoms				+	+		+				3
Pulmonary symptoms			+				+				2
BSR > 20 mm/h	+	+	+	+		+	+	+	+	+	9
Hb 10 g/100 ml	+	+		−		+			+	+	6
Leukocytes > 10.000	+		+	+	+				+		5
Eosinophils > 5%	+			+						+	3
NPN > 50 mg/100 ml				+		+					3
Proteinuria	+		−	+		+		+		+	6
Haematuria						+					1
Alive after 10 years	Yes	Yes	No	N	Yes	N	N	Yes	Yes	No	5
Survival time after diagnosis (y)			< 1	2		6	1			8	

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MYOCARDIAL INFARCTION IN MALMÖ 1960–1968

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Abstract. Malmö is unusually well suited for studies on the frequency of disease since the whole population of about a quarter of a million inhabitants is served by a single hospital. The case records from the Department of Medicine were perused and those with a definite diagnosis of acute myocardial infarction (AMI) were collected. The hospital material comprised 3 249 patients with a mean age at onset of 67 years (65 years for the 2 166 males and 72 for the 1 083 females). The *incidence was found to increase* with age, the rise being more pronounced in the higher than in the lower decades. There was an over-representation of patients with diabetes mellitus, and mortality was higher among these patients than in the whole hospital material. The hospital mortality for AMI was 33.1%: 30.4 for men and 38.5 for women, the latter higher figure being due to the higher mean age of the women. Rather pronounced yearly differences in mortality were observed, with a low of 27.9% in 1964 and a high of 39.9% in 1966. Thirty-five per cent of the patients arrived at the hospital within 3 hours after onset of symptoms, but the mean delay was 31 hours. The mortality figure of 33.1% is misleadingly low because many patients die before they reach hospital. Including patients who died at home and were autopsied in the Department of Forensic Medicine, and those who were dead on arrival at the hospital, the mortality rose to 43%. The importance of preventive measures as a complement to the coronary care units is stressed.

Malmö is unusually well suited for studies on the frequency of a disease since the whole town—256 000 inhabitants in 1968—is served by a single hospital. This feature has been used in previous studies (1, 3, 6, 17). In connection with the establishment of a coronary care unit in 1968 for part of the Malmö patients with acute myocardial infarction (AMI) we thought it worthwhile to obtain some epidemiological data as a background for further developments in this field. The present study starts where the previous investigation left off (1, 17) and comprises the nine years from 1960 to 1968.

METHODS AND MATERIAL

The case files from the Department of Medicine were perused and those with a definite diagnosis of AMI were collected. It has been shown (17) that almost all acute infarctions are treated at the *Medical Department. The diagnostic criteria* were mainly those described by Sievers (17), but we also utilized the enzymatic response, including serum glutamic oxaloacetic acid transaminase (GOT) and, from 1964, lactic dehydrogenase (LDH).

Several variables were extracted from the records following a schedule worked out by Dr T. Lundman at the Serafimerlasarettet, Stockholm. This was done to permit comparisons with other hospitals and to make the values easily accessible for data processing. The relevant data in numerical form were subsequently transferred to punch cards for evaluation by a computer.

Obesity was defined according to the criteria published by Paasikivi (14). The patients were judged as hypertensive if the diastolic pressure was repeatedly 105 mmHg or more or if the patient received antihypertensive treatment. Typical angina elicited by effort or cold on more than one occasion was necessary for a diagnosis of angina pectoris. Angina starting a few days before the infarction was included. Cardiac decompensation was diagnosed when the patients had clearcut symptoms of edema, dyspnea and cyanosis or were treated with digitalis and/or diuretics. GOT was determined according to the method described by Levine and Hill (11) and LDH by Schwartz et al. (16).

The hospital material refers to the 3 249 patients treated in the Medical Department during 1960–1968.

The total material refers to the same period but, in addition to the hospital material, it also includes other persons known to have died from an AMI. To this group belong patients referred to the emergency ward, where they died, and persons who were dead on arrival at the emergency ward. The diagnosis had been verified in all these cases by autopsy. Furthermore, the files of the Department of Forensic Pathology were checked and persons who had died at home in an AMI were also included in the total material. In these cases, too, the diagnosis had been verified by autopsy.

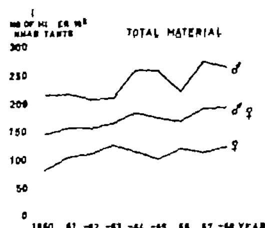


Fig 1 Total material. Incidence of AMIs in Malmö calculated as number of infarctions per 100 000 inhabitants per year

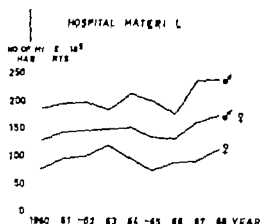


Fig 3 Hospital material. Incidence of AMIs in Malmö calculated as number of infarctions per 100 000 inhabitants per year

RESULTS

A. *Total material*

The total material comprises 3 819 persons, of whom 2 538 were males and 1 281 females. The mean age at the infarction was 67.45 years 65.08 for males and 72.15 for females. There is a tendency for the mean age to increase with time, being 64.96 years for men in 1960 and 65.49 in 1968 the corresponding figures for females being 69.75 and 74.09 years. Persons not treated in hospital constituted 15 of the total material and a further 28% died in hospital, making a total mortality of 43 Of the 15% not admitted to a hospital ward 67% were dead on arrival at the emergency ward 3 % died at home and 1% in the emergency ward. No sex differences were found in this respect.

There is a steady increase in the number of AMIs throughout the sequence of years, even when the incidence is calculated per 100 000 inhabitants (Fig. 1) This applies to both sexes. Table I gives the age distribution for the total material and the sex ratio.

B *Hospital material*

Sex and age distribution. The hospital material comprises 3 249 patients, of whom 2 166 were males and 1 083 females. The mean age at the infarction was 67.03 years, 64.57 for males and 71.96 for females. The conformity with the total material is obviously good. There is a tendency for the mean age at infarction to increase, 65.81 years in 1960 and 67.91 years in 1968 (for males 63.98 and 64.80 for females 69.89 and 74.12 years)

Fig. 2 shows the ratio of men to women during different years in the hospital material. The sex ratio was similar to that in the total material, with a figure of 9.9 for the age group 30–49 5.2 for 50–59 2.6 for 60–69 1.1 for 70–79 and 0.8 for the age group 80–99 with a mean male/female ratio of 2.0 for the whole hospital material during 1960–1968.

Incidence The increase in the number of AMIs throughout the years 1960–68 is shown in Fig. 3. The rising trend applies to males as well as to females. In Fig. 4 the material is broken down by

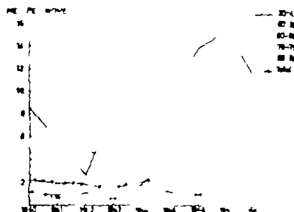


Fig 2 Hospital material. Sex ratio in the different age groups and for all the patients each year

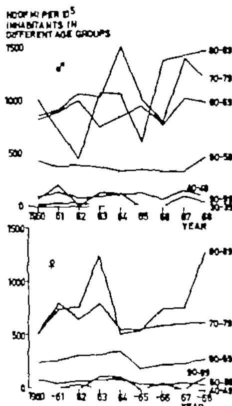


Fig. 4. Hospital material. Incidence of infarctions per year and in different age groups in men and women, respectively

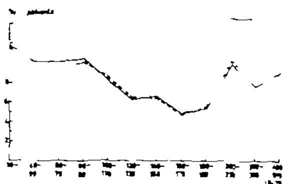


Fig. 5. Hospital material. Maximal GOT values in male, female and all patients.

decades. The rising incidence is not more pronounced in the lower than the higher age groups. The trend is similar for both sexes. The incidence of AMI is lower in the highest decade (90–99 years) than in the 80–89 decade and this applies both for males and females.

Previous history. Obesity according to the criteria used in this study was present in 308 of 2 166 male patients (14.2%) and in 211 of 1 083 female (19.5%)

A diagnosis of diabetes mellitus had been made in 165 of the male (7 6%) and 137 of the female patients (12.7%) Both these figures are higher than the incidences of 2.1% for males and 2.8% for females below 80 years reported by Nilsson et al. (13) for the general population.

A history of hypothyreosis occurred in 12 of the male (0.6%) and 10 of the female patients (0.9%) but hyperthyreosis was more common, being found in 17 of the male (0 8%) and 25 of the female patients (2.3%) Oophorectomy had been performed in 54 of the female patients (5.0%) A total number of 1 356 oophorectomies were performed in Malmo during the years 1961 through 1968, resulting in 2% oophorectomies in women 40 years of age or above

Hypertension was diagnosed in 154 of the male (7 1%) and 164 of the female patients (15 1%) Angina pectoris occurred in 585 of the males (27 0%) and 252 of the females (23.3%). A combination of these two symptoms was found in 122 of the male (5 6%) and 160 of the female patients (14 8%).

Only 7 of the male and 6 of the female patients presented a previous history of valvular heart disease. Cardiac decompensation was more common, occurring in 203 and 220 patients, respectively. A combination of valvular heart disease and cardiac decompensation was found in 5 of the male and 9 of the female patients.

Clinical symptoms. Chest pain was the most common of the symptoms that brought the patients to the hospital, it occurred in 66.9% of the

Table I. *Total material. Age distribution for 1960–1968 in total number of infarctions and per cent of the total material* (=3 819).

Age group (y)	AMI No.	AMI %	Men/women
20–29	18	0.5	
30–39	26	0.7	24/2 12 0
40–49	219	5.7	199/20 10 0
50–59	709	18 6	587/122 – 4.8
60–69	1,219	31 9	881/338 – 2 6
70–79	1 133	29 7	615/518 – 1.2
80–89	463	12.1	206/257 0 8
90–99	32	0 8	17/15 – 1 1

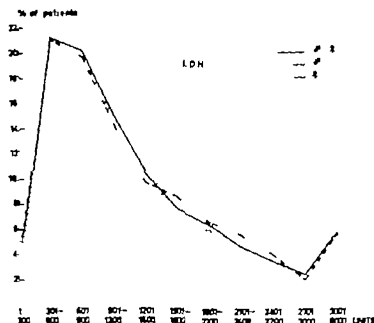


Fig 6. Hospital material. Maximal LDH values in male, female and all patients.

male and 55.6% of the female patients. Next came dyspnea in 12.8 and 16.5% while pulmonary edema only occurred in 3.4 and 5.6% of the male and female patients, respectively. Tiredness was the first symptom in 1.1% in both sexes, while syncope occurred in 4% of the male and 3.3% of the female patients. Nausea and vomiting as the starting symptom was found in 11.5% of the male and 14 of the female patients.

Enzyme values The distributions of the maximal GOT and LDH values are shown in Figs. 5 and 6. The tendency to a greater number of male compared to female patients with high GOT values has no counterpart in the LDH values. The percentage distribution of GOT and LDH values is fairly constant throughout the years 1960–68 indicating that the severity of the disease has been fairly constant during this period.

Clinical complications Shock was reported to occur in 205 of the male (9.5) and 119 of the female patients (11.0) during their stay in hospital. Corresponding figures for pulmonary edema were 156 (7.2%) and 103 (9.5). Arrhythmias were observed electrocardiographically in 29%. Thromboembolism was reported only for 34 patients less often in female (0.6) than in male (1.9%). No tendency to increased severity of the infarction, as judged from the number of complications, was seen during the years 1960–68. Resuscitation was performed in 268 patients, with a successful result in 27. In a further 25 patients resuscitation was primarily successful but they were not discharged, because 21 with cardiac restitution died later in the hospital, as did 4 others with both cardiac and cerebral restitution.

Mortality The hospital mortality among all 3 249 patients amounted to 33.1% (30.4 for the men and 38.5 for the women). Fig. 7 shows the hospital mortality rates in different age and sex groups.

It should be kept in mind, however that when compared to the number of females the number of males in a population steadily diminishes with increasing age. After correcting for this, it is clear that the mortality from AMI is higher in males than in females with increasing age. This is shown in Table II where mortality has been calculated as the number of males and females per 100 000 inhabitants who died from AMI in different decades.

Is there any consistent change in mortality during the years 1960–68 indicating a change in the severity of the disease? Fig. 8 shows that there are rather pronounced variations from year to year with a low of 27.9% in 1964 and a high of 38.9% in 1966, but there is no consistent trend up

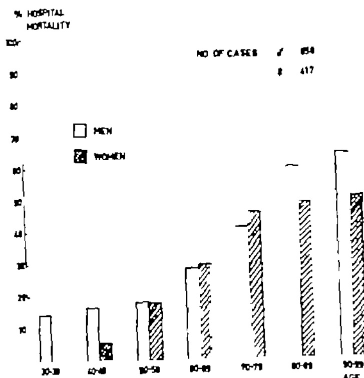


Fig. 7. Hospital material. Percentage hospital mortality in 2 166 men and 1 083 women in different age groups. No. of cases denotes the number of patients on which the percentage figures are based.

or down over the period as a whole. Fig. 9 presents the material broken down by decades.

A large proportion of the patients with AMI die soon after admission to hospital, especially during the first 6 hours (Fig. 10). In the hospital material 195 patients out of 1 062 (18.4%) died within the first 6 hours of their stay while 6.7% died within 6–12 hours, 3.0% within 12–24 hours and 71.8% during the remaining time in hospital. The proportion dying within the first 6 hours was slightly higher among the male patients, 20.3% than among the female 15.4% while the opposite applied to mortality after the first day 70.0 and 74.8% respectively.

The mode of death is mentioned in the hospital records in 918 cases and was ascribed to shock in 27.6, pulmonary edema in 13.8 combination of shock and pulmonary edema in 4.7 arrhythmia in 19.8 and mors subita in 34.1%. The percentage distribution between the modes of death is rather constant over the years except for 1968 when deaths in arrhythmia amount to 33.1% as against 17.6% during 1960–67 while deaths denoted as mors subita decreased to 20.3% as against 36.4% during 1960–67.

Postmortem observations

The autopsy rate in Malmö decreases with age but is comparatively high and rose from 69% in 1961 to 89% in 1968. Only 19 of the 1 062 patients who died of their AMI were not autopsied. In 765 patients (72%) coronary thrombosis was found and in 32 (3%) a rupture of the myocardium. Neither thrombosis nor rupture was reported in 255 of the patients (24%). Anticoagu-

Table II. *Hospital material. Number of male and female patients dead in AMI*

See further the text

Age (y)	Men	Women	Men/women
30–39	2.1		
40–49	18.9	0.6	31.5
50–59	66.7	11.3	5.9
60–69	234.6	75.4	3.2
70–79	442.8	278.4	1.6
80–	61.3	36.7	1.7

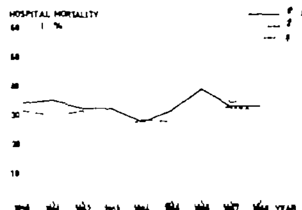


Fig. 8 Hospital material. Hospital mortality in men, women and all patients during each year.

lants are routinely used the number with emboli or thrombi in peripheral vessels was reported to be low only 4 patients, 0.4%—and only 2 patients, 0.2% were reported to have pulmonary emboli.

Some temporal data. The mean stay in hospital was 20.74 days, S.D ±2.95 and was slightly shorter for men 19.99 S.D ±2.82, than for women 22.23 days, S.D ±3.19 There is a trend

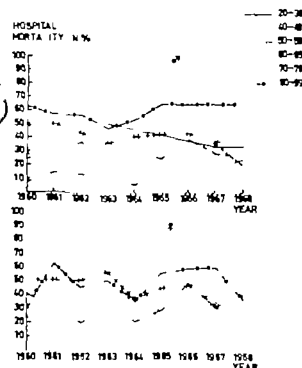


Fig 9 Hospital material. Hospital mortality in men and women in different age groups during each year

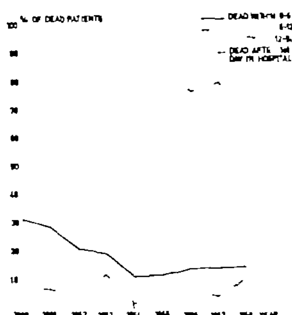


Fig 10 Hospital material. Mortality during different parts of stay in hospital.

to a shorter stay in hospital over the years, with a mean of 21.29 days, S.D ±3.52 in 1960 and 19.16 days, S.D ±3.06 in 1968 This applies to both sexes, corresponding figures for men being 19.93 and 17.66 days and for women 24.35 and 22.16 days.

The delay from the onset of symptoms until arrival at hospital averaged 30.95 hours, 30.66 for men and 31.60 for women Thirty-five per cent arrived at hospital within 3 hours after onset of symptoms but 21% more than 4 days after onset. This delay become more pronounced with a mean of 20.41 hours, S.D ±3.87 in 1960 as against 27.04 S.D ±4.35 in 1968 The longest delay was observed in 1966 37.92 hours, S.D ±4.80. This tendency also applies to both sexes.

Fig. 11 shows the number of infarctions per 2 hour periods of the day in the cases where this could be traced. There seems to be a certain connection with work, as the number of infarctions is higher during the active part of the day The rate is lower during lunchtime and dinnertime and higher between 6 p.m. and 10 p.m when overtime work is done. Furthermore it is higher between 2 and 4 a.m. when the blood pressure (BP) is usually low

The time of death is shown in Fig. 12. More patients died from their AMI during the day than during the night. This is more pronounced for pa-

Fig. 11 Hospital material. Number of patients with an AMI divided according to time of onset. The figures denote the mean for all years 1960–1968.

tients dying in hospital—hospital material—than for persons dying outside the ward—total material minus hospital material.

Seasonal aspects. The number of AMIs is distributed fairly evenly over the months of the year though it tends to be higher in winter than in

Fig. 12. Hospital material. Time of death from 00.00 to 24.00 hours. Sex distribution is shown for hospital material and for patients who died outside hospital (total material minus hospital material).

Fig. 13 Hospital material. Infarction rate in % of patients in men, women and all patients per month, correlated with (*a*) the mean temperature, the difference between the highest and the lowest temperature and (*b*) the precipitation (measured as mm per month and as days with precipitation). The values for all the variables are the mean for each month during all the years 1960–1968.

summer. Fig. 13 shows the infarction rate for all the years 1960–68 in relation to some meteorological data. The lowest rate is found in August. The mean temperature in June is the same as in August, but the infarction rate is higher and similar to the rate in February when the mean temperature is much lower. The difference between the highest and lowest temperature is much greater in

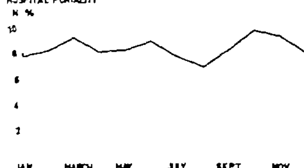


Fig 14 Hospital material. Mortality rate in % of patients during each month of the year

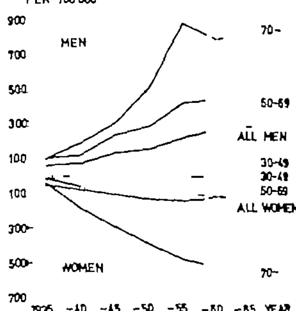


Fig 16. First infarct material. Incidence of hospitalized first infarcts in men and women of different ages per 100 000 same-sexed, same-aged inhabitants of Malmö in different periods from 1935 through 1968. See also the legend to Fig. 15.

April than in February and March, and yet the infarction rate does not differ very much. Neither does there seem to be any connection between precipitation (measured in mm per month or in days with precipitation) and the infarction rate.

The solar activity displays a pronounced cyclic pattern. The mean solar activity for each of the years 1960–1967 was compared to the incidence of AMI and the mortality figures. Detailed data will be presented elsewhere.

In Fig. 14 th mortality over the years is given for each month f the year

C. First far t ma.erial

First nfarct ns constituted 77% of the hospital material, second infarctions 18% third 4 fourth 0.8 and fifth infarctions 0.2% The distribution was similar for men and women.

By selecting the first infarcts it is possible to make a direct comparison with the Malmö material previously published by Sievers (17). Fig. 15 shows the sex ratio throughout the years. The incidence shows a steady increase as displayed in Fig. 16. As in the 1935–1959 material, the 1960's show a trend towards a higher age for the onset of the first infarct, though the curve is levelling off at least for men (Fig. 17). The hospital mortality level has remained fairly constant around 32% but with rather pronounced variations above and below this level from year to year (Fig. 18).

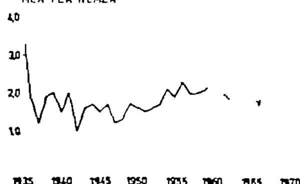


Fig 15 First infarct material. Men women rate among acute first infarctions in Malmö 1935 1968. — refers to Sievers' material (17) 1935–1959 – refers to present material 1960–1968.

DISCUSSION

By combining the present study on the frequency of AMI with a previous one by Sievers (17) it is possible to follow the incidence of hospitalized first infarcts from 1935 through 1968 This has been done in Fig. 16, with a correction for the population increase. There is clearly an increase in the incidence but it should be kept in mind that many factors may have a bearing on this result besides a true increase It is noteworthy that in the period 1935–1959 the use of the ECG technique, including the number of leads, increased as did

the diagnostic ability Furthermore, serum GOT was employed in the period 1960–1968 but not in the previous period, and serum LDH was introduced in 1964

The mortality for first infarcts in the years 1935 through 1968 is shown in Fig. 18. There is a slight decrease from an average of 34 6% in the period 1935–1959 to 29 6% in 1960–1968. This can of course be a result of improvements in the treatment of patients. This view might be supported by the fact that the mean age of the patients is steadily increasing and that, since the mortality rises with age one would rather expect an increasing mortality But there are other possible explanations. Malmö is steadily growing and, since the mortality in AMI is highest during the hours immediately following the onset of the disease, it is possible that more patients die because of longer transportation, before they arrive at the hospital this would result in a selection those being hospitalized having the best prognosis. Another possibility is that the disease has become more benign. Furthermore our diagnostic tools have improved, resulting in a more precise diagnosis, so that many cases which were previously classified as possible infarctions now get a definite diagnosis thus more benign cases are included in the material. Finally resuscitation has had some though rather a small, influence out of 3 231 AMIs, resuscitation was started in 268 patients but only 27 of these were discharged from hospital. By way of comparison it may be mentioned that in the coronary care unit, which was well established in 1969 13 patients were electroconverted because of ventricular fibrillation and 7 of them survived.

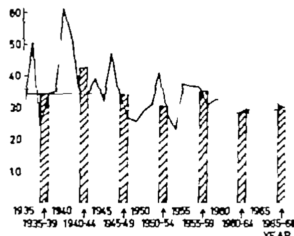


Fig. 18 First infarct material. Hospital mortality per year (curve) and 5-year period (bars) See also the legend to Fig. 15 The horizontal lines denote the mean mortality for the period 1935–1959 (—) and 1960–1968 (– –)

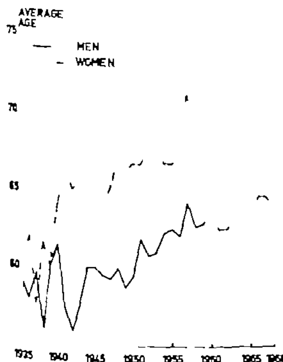


Fig. 17 First infarct material. Average age of men and women having first infarcts in different years. See also the legend to Fig. 15.

The hospital mortality of 29 6% for first infarcts in the years 1960 through 1968 is high but, of course does not give any indication of the true mortality This also applies to the mortality figure of 33 1% in the hospital material Since it is known that many patients die before arrival at the hospital, I tried to identify these by including patients who died at home or were dead on arrival at the emergency ward (see Methods and Material) By including these patients, in all of whom the diagnosis of acute infarction was verified by autopsy the mortality figure rose to 43% This figure is also an underestimate since, even if the autopsy rate in Malmö is high (see further Results) some patients were not autopsied and some patients had such a fresh infarct that it was probably missed at a routine autopsy

To overcome these difficulties I calculated with A. M Bolander's help the mortality from the death certificates compiled for the Official Statistics of Sweden including patients both with and without a verification of the diagnosis at autopsy It was found that, during the period 1961 through 1968 2 760 patients had died from an AMI (420.1) according to the death certificate diagnosis, resulting in a mortality of 59% In these calculations the percentage was based on the 1952 discharged patients in the hospital material. The results based on death certificates are somewhat uncertain and may give a falsely high mortality figure On the other hand the mortality figure of 43% based on the total material (see Methods and Material) including patients dead before hospitalization with the diagnosis verified by autopsy is definitely too low and the true mortality will be somewhere in between Kuller (9) concluded that approximately 25–30% of patients with incident cases of myocardial infarction die suddenly and for a cohort of myocardial patients with myocardial infarction there are as many deaths within the first 24 hours as during the next 5 years. Kuller also found from a number of studies that, in about 70% of all deaths due to arteriosclerotic heart disease the individuals died outside of a hospital or were dead on arrival.

The introduction of coronary care units was a major step towards reducing the mortality in AMI although only a few well-controlled studies ha e been published (5 7) However although a number of patients ha e been rescued by the coronary care units, there is still a substantial mortality caused by pump failure because of an extensive myocardial infarct. Furthermore many patients die from their infarction or coronary heart disease before arrival at hospital It is apparent, then that the concept of coronary care units has to be combined with the concept of primary and/or secondary prevention if the mortality in coronary heart disease is to be further reduced. This is still more apparent in iew of the long delay from the onset of symptoms until arrival in hospital although 35% in the present material arri ed within 3 hours after onset, the mean delay was 20 hours and 21% arri ed more than 4 days after onset. Moss and Goldstein (12) analyzed hospital arrival time and found that the longest delay was not caused by transportation, which accounted for only 14% but by the decision time, i e. the interval between the onset of acute coronary symptoms and the patient's decision to seek medical help which made up 51% of hospital arrival time.

A recent WHO report (10) claims that the death rates from arteriosclerotic and degenerative heart disease in the age group 45 to 54 years had increased considerably in most countries between 1955 and 1967 The highest increase 66% was found in Holland, while Japan was the only country to show a decrease 14% Sweden showed an increase of 15% (♂+25% ♀−22%) In the present material there is also an increase in morbidity more pronounced for men than women (Figs. 4 and 16) This increase is by no means restricted to the younger age groups, being at least as pronounced in the higher age groups. It is also apparent that there are very pronounced variations in the mortality figures from year to year As shown in Fig. 8 referring to the present study from 1960 through 1968 there was a low of 27.9% in 1964 and a high of 38.9% in 1966 and these figures were based on a fairly large number of infarctions, approximately 350 patients per year It is obvious that the evaluation of therapy for acute infarction by comparing results in consecutive years must be based on much larger materials. If smaller materials are used they must have precisely matched controls.

It is well known that high blood lipid levels, increased BP smoking and a sedentary life are more often found in patients with a myocardial infarction than in those showing no signs of coronary heart disease Other risk factors ha e also been discussed. In the present material a diagnosis of diabetes mellitus was found in the hospital material in 7.6% of the male and 12.7% of the female patients. There is no study of the frequency of diabetes mellitus in Malmö but data are available from the county of Kristianstad, which is situated close to Malmö. According to these studies, diabetes mellitus is to be expected in 2.1% of the males and 2.8% of the females below 80 years of age (13) The incidence of diabetes mellitus in the present infarction material from 1960–68 was thus four times higher than in the Kristianstad survey regardless of sex. Not only was the frequency of diabetes higher in the infarction material when compared to the general population but the mortality was higher among the diabetics, 45.4% (♂ 41.2% ♀ 50.4%) corresponding figures in the hospital material were

33.1% (♂ 30.4% ♀ 38.5%). The mean age of the diabetics was 68.51 years (♂ 65.77 ♀ 71.80); corresponding values in the whole hospital material ere 67.03 years (♂ 64.57 ♀ 71.96).

A history of hypothyreosis was found in 22 patients and of hyperthyreosis in 42. The figures are small but it is noteworthy that hyperthyreosis, which decreases th. serum cholesterol level, was more common than hypothyreosis, which increases it. This could indicate that if a heart with moderate coronary heart disease and no symptoms when coping with a normokinetic circulation, is faced with the hyperkinetic circulation of the hyperthyreotic state, the result will be deleterious.

There are reports in the literature claiming a relation between the incidence of coronary heart disease and the en ironmental temperature (2, 4, 15). Other authors have been less convinced of the relation (2). In the present material I have tried to compare the infarction rate to certain meteorological data (Fig. 13). Even if there is some relation between the mean temperature of the month and the mortality and incidence of infarctions, the correlation is by no means strong. It may be that the present material is too small. But it should also be kept in mind that there are other circannian rhythms than the temperature, e.g. the serum cholesterol level, which shows a peak in January compared with June (8). Furthermore the present study refers only to patients with an AMI, while some of the previous studies include all patients with coronary heart disease. Other meteorological parameters such as precipitation and the difference between the highest and lowest temperature of the month did not show any correlation to the incidence or mortality of acute infarction. Neither was it possible to obtain any relation between the incidence of infarctions and sun flare activity. There seemed, however, to be a circadian relationship, with a larger number of infarctions during the acti e part of the day (Fig. 11).

ACKNOWLEDGEMENTS

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LOCALIZED SUPRAVALVULAR AORTIC STENOSIS COMBINED WITH MENTAL RETARDATION AND PECULIAR FACIAL APPEARANCE

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Abstract. Three patients are reported who were admitted to hospital because of peculiar elfin-like facial appearance, mental retardation and systolic diamond-shaped murmur recorded on phonocardiography. These findings are known to be connected with late infantile hypercalcemia, supravalvular aortic and pulmonary arterial stenoses. Two of the patients had membranous supravalvular aortic stenosis, while the third had coarctation of the aorta combined with subclavian and pulmonary artery stenoses. In one patient with membranous supravalvular aortic stenosis no pulmonary artery stenosis could be found. All three patients displayed bicuspid aortic valves. The findings in this anomaly are therefore more complex than generally described in the literature. Relevant to the bicuspid aortic valves is probably an early-diastolic decrescendo murmur recorded on phonocardiography.

In 1952 Fanconi and Girardet, together with Schlesinger (3) reported two patients with infantile hypercalcemia, mental retardation, peculiar facial appearance, and systolic murmurs regarded as innocent. Rashkind (7) reported 52 patients with infantile hypercalcemia collected from the literature, 33 of whom had significant cardiac or vascular murmurs. Since then reports have been published of patients with supravalvular aortic stenosis combined with arterial stenoses located peripherally in the aorta, in the systemic and the pulmonary arteries, combined with mental retardation and an elfin-like facial appearance.

In a series of 68 cases of supravalvular aortic stenosis which Peterson et al. (6) had collected mostly from the literature, 23 cases had mental retardation and 16 a peculiar elfin facial appearance as well. In 1964 Beuren et al. (1) reported 10 patients with supravalvular aortic stenosis diagnosed by the authors during a period of 1 1/2 years, all with pulmonary artery stenosis, dental anomalies and with elfin-like faces. Aortic valvular malformation has also been reported in similar patients (2, 7).

Table I. *Three cases with supravalvular aortic stenosis combined with mental retardation and peculiar facial appearance*

EDM = end diastolic murmur. LV = left ventricular.

Pat. no.	Age (y)	Sex	Symptoms	Murmurs	ECG	Location of stenoses	Peak systolic gradients (mmHg)	PCV pressure on exertion (mmHg)
1	10	♀	Tires easily. No dyspnoea	Systolic diamond-shaped. Short EDM	Right axis deviation. LV hypertrophy	Supravalvular Membranous	20	
2	18	♂	Slight exertional dyspnoea	Systolic diamond-shaped. Short EDM	Right axis deviation. LV hypertrophy	Supravalvular Membranous	60	15
3	18	♂	Slight exertional dyspnoea and angina pectoris	Systolic diamond-shaped. Short EDM	Right and left ventricular hypertrophy	Coarctation of aorta. Left subclavian. Pulmonary arterial	40 25 & 40 20	18

This report concerns three patients with mental retardation, elfin-like faces regarded as typical of late infantile hypercalcemia and combined with arterial stenoses at various systemic and pulmonary locations.

MATERIAL AND RESULTS

Three patient re reported, all of hom had a peculiar lfin-like facial ppearance (Fig. 1), mental re ardation and diamond-shaped systolic and short early-diastolic murmurs One as referred from pediatric department t the age of 5 years, and heart catheterization was pe formed t the age of 10 years The remaining two patients ere referred f om the State Rehabilitation Center of Oslo t the ge f 18 years

All had been subjected to a routine clinical examination including left and right he rt catheterization with ardioangiography (Table 1).

The ymptoms ere moderate One became readily tired on xertion but had no dyspnoea, and one had even angina pec oris slight to moderate exertion. ECG registration sh ed right axis deviation in all cases, left ventricular hypertr ph in two, and one had signs of combined ventricular h pertrophy

The angiocardiographic tudy showed that all had bicuspid aortic valves (Figs —4) and, in addition, tw had membranous supravalvular stenosis (Figs. 2 nd 3). The last patient had moderate coarctation of the aorta but no stenosis immediately bove the aortic valves (Fig. 4).



Fig. 1. Case 1. A girl, 10 years of age with membranous supravalvular aortic stenosis, mental retardation and an "elfin-like" facial appearance. Note the broad lips, the broad protruding tip of the nose and the divergent squint of the left eye.

This patient also had multiple pulmonary and bilateral subclavian arterial stenoses (Fig. 5). Peripheral pulmonary arterial stenosis was absent in one patient, in the third patient pulmonary angiography was not performed.

The peak systolic gradients across the arterial stenoses ere slight to moderate. The two patients ith membranous supravalvular aortic stenosis had gradients of 20 and 60 mmHg, and in the patient with peripheral arterial



Fig. 2. Case 1. Left ventricular angiography shows ( ) in the AP view the supravalvular aortic sten sis (large arrow) and bicuspid aortic valves (small arrow) (*b*) In the lateral view the supravalvular stenosis appears to be membranous (arrow) and (*c*) in diastole the membrane (large arrow) and the bicuspid valves (small arrow) are seen in the lateral view



Fig. 3. Case 2. Angiography of the aortic root show (*a*) in the left anterior oblique view small septal indentation (arrow) immediately above the orifice of the left coronary artery while (*b*) in the lateral view the septum is seen across the entire aortic root. The supravalvular membrane is semilunar and located postero-laterally above the left coronary artery

stenosis the gradient across one of the pulmonary arterial stenoses was 20, across the coarctation 40 and across the subclavian arteries 25 and 40 mmHg.

COMMENTS

The three patients reported are remarkable in that all had a peculiar and strikingly similar "elfin-like" facial appearance combined with a slight to moderate convergent squint, dental anomalies, broad lips and mental retardation (Fig. 1). These findings have been known to be connected with late infantile hypercalcemia (4) and supravalvular aortic stenosis (5–8). Pulmonary and systemic arterial stenoses have also been described (1).

In the series of ten patients reported by Beuren et al. (*1*) all had supravalvular aortic stenosis and pulmonary arterial stenosis. Among the three patients reported here pulmonary arterial stenosis was present in one. In another no pulmonary arterial stenosis could be seen.

It is also of interest to note that one patient had no supravalvular aortic stenosis (Fig. 4), but instead a moderate coarctation of the aorta.

In the two patients with supravalvular aortic



Fig. 4. Case 3. Angiography of the aortic root show (*a*) bicuspid aortic valves but no supravalvular stenosis and (*b*) in the AP view coarctation of the aorta is seen at the usual location (arrow)



Fig. 5. Case 3. (*a*) Angiography of the aortic arch shows stenosis (white arrow) and an unusual tortuous course of the left subclavian artery (black arrow). (*b*) Pulmonary angiography shows multiple stenoses (arrowheads) in both pulmonary arteries with poststenotic dilatations.

stenosis this was found to be membranous and presented only a slight to moderate systolic gradient across the stenosis. On the other hand all patients had bicuspid aortic valves.

The combination of findings in this anomaly therefore appears to be more complex than described in the literature (1, 6, 7).

In addition to systolic diamond-shaped murmurs recorded in all three patients on phonocardiography, a short early-diastolic decrescendo murmur was also noted. Relevant to this is presumably that the aortic valves in all three cases were found to be bicuspid. Aortic valvular anomalies have been described in combination with supravalvular aortic stenosis in similar patients (2, 7). An alternative explanation to this early-diastolic murmur may be the early-diastolic reflux of blood in the central aorta, causing a murmur in the stenotic area. However, the same murmur was also present in the patient without supravalvular aortic stenosis but with coarctation of the aorta.

The patient with coarctation of the aorta presented a differential-diagnostic problem. The combination of signs of left ventricular hypertrophy in the ECG and normal blood pressures measured in both arms and legs strongly suggested a stenosis centrally to the aortic arch. In this patient the combination was, however, brought about by bilateral subclavian arterial stenoses (Fig. 5) combined with stenosis at the usual site of coarctation of the aorta (Fig. 4).

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METABOLISM OF CHOLIC ACID 24-^{14}C IN PATIENTS WITH HEPATOBILIARY DISEASES

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Abstract. Cholic acid 24-^{14}C was injected i.v. into 5 patients with chronic liver disease (four with Laennec's cirrhosis and one with amyloidosis) and three patients with acute liver failure of different etiology (massive hepatic necrosis of unknown etiology, carbon tetrachloride liver injury, infectious hepatitis). The rate of disappearance of isotope from the blood during the first 60 min was depressed in patients with icterus. An anicteric patient with amyloidosis showed greatly depressed removal rate. Only trace amounts of isotope were excreted in urine during the first hours following injection and only a small fraction of the cholic acid pool was eliminated by urinary excretion, since less than 13% of the administered isotope was excreted in urine during the four days following injection. Similar studies were performed in two patients with interrupted enterohepatic circulation of bile acids due to calculi in the choledochus. Bile was sampled through a T-tube inserted in the choledochus. These patients had impaired liver function, as indicated by bilirubin concentration above 6.0 mg/100 ml. The injected isotope was rapidly excreted in bile and all isotope was recovered in bile within 24 hours in conjugated form. Chromatographic analysis of urinary labelled metabolites showed that eight of the ten patients mainly excreted labelled conjugated bile acids. In two of the patients, one with Laennec's cirrhosis and one with hepatic necrosis, a constant excretion of 30–40% of unconjugated labelled bile acids was observed.

An increased level of total serum bile acids is regularly observed in patients with liver diseases with elevated serum bilirubin (2, 10). In obstructive jaundice the increase is mainly due to elevation of conjugated bile acids in serum, whereas in infectious hepatitis or in cirrhosis the state of conjugation of serum bile acids varies (7). Using i.v. injections of tracer doses of isotopic cholic acid, it has been demonstrated that there is a very slow isotope disappearance from serum in patients with Laennec's cirrhosis with and without jaundice (1) and in jaundiced patients with infectious hepatitis (11).

The urinary excretion of bile acids in jaundiced patients has been demonstrated (1, 12). In patients with Laennec's cirrhosis 9–67% of injected cholic acid 24-^{14}C was recovered from the urine during the seven days following administration (1) and in patients with viral hepatitis 3–35% of injected cholic acid ^{3}H was recovered from the urine during the four days following administration (11).

In the present investigation cholic acid 24-^{14}C has been injected into patients with hepatobiliary diseases and the rate of removal of isotope from serum and the urinary loss of isotope have been followed. The extent of conjugation of the labelled urinary bile acids was determined to find out whether the different liver diseases were associated with changes in the activity of the bile acid conjugating and deconjugating enzyme systems.

MATERIAL

Diagnoses, ages, and liver function tests of the 10 male patients studied are listed in Table I and abbreviated case reports are given below.

During the investigation patients E and F had biliary drainage through T-tubes inserted into the choledochus. The major portion of the bile was excreted through the catheters due to calculi obstructing the distal part of choledochus.

CASE REPORTS

Case A

Man aged 71. Heavy drinker. Three years earlier treated in hospital for cirrhosis of the liver. Now admitted to hospital with increasing liver insufficiency. At first some improvement, but 18 days after admission severe hematemesis and death ensued. Autopsy revealed far advanced cirrhosis of the liver of portal type, esophageal varices and massive gastro-intestinal hemorrhages, and extra-medullary

Table I. *Clinical information, liver function test results and initial serum clearance of cholic acid 24-^{14}C in the male patients studied*

– = not observed, + = observed

Pat.	Age (y)	Diagnosis	Ascites	Esophageal varices	Gastro-intestinal hemorrhage	Bilirubin total (mg/100 ml)	Alkaline phosphatases (U)	GPT (U)	GOT (U)
A	71	Cirrhosis Laennec's	+	+	+	4.0	9	48	52
B	51	Cirrhosis Laennec's	+	+	+	18.5	18	32	54
C	60	Cirrhosis Laennec	+	–	–	2.7	5	38	90
D	41	Cirrhosis Laennec	–	–	–	1.8	9	46	50
E	56	Chronic cholecystitis with cholelithiasis	–	–	–	6.0	35	320	44
F	58	Chronic cholecystitis with cholelithiasis + hepato-renal syndrome	–	–	–	9.0	10	46	14
G	60	Massive hepatic necrosis	–	–	+	7.2	5	6 200	12 800
H	55	Carbon tetrachloride liver injury	–	–	–	3.0	5	4 400	4 [illegible]40
I	70	Amyloidosis	–	–	–	0.7	56	34	58
K	19	Infectious hepatitis	–	–	–	12.9	14	3 100	2 000
	20–40	Normal values				< 1.2	< 8	< 35	35

blood formation in the spleen. Beginning of metabolic study five days after admission.

Case B

Man aged 50. Earlier heavy drinker. Admitted to hospital one month after onset of signs of increasing liver insufficiency. The patient became progressively emaciated and died on the 1[illegible]th day with signs of hepatic coma and gastro-intestinal hemorrhage. Autopsy revealed advanced cirrhosis of the liver of portal type, pancreatitis, varices in esophagus and stomach and massive hemorrhage into the gastro-intestinal tract from ruptured varicose vessel. Beginning of metabolic study (and antibiotic treatment, neomycin 2 g 3 times daily) three days after admission.

Case C

Man aged 60. For the last five years treated in hospital several times for chronic alcoholism and cirrhosis of the liver (two years earlier delirium tremens). Admitted to hospital for bronchopneumonia and signs of liver insufficiency. X-ray revealed bronchopneumonia. Treated with penicillin and discharged improved after three weeks. Beginning of metabolic study three days after admission.

Case D

Man aged 41. History of heavy drinking. For the last three years polyneuritis. Admitted to hospital for investigation and rehabilitation. Liver enlarged, liver tests slightly pathological. Liver biopsy revealed pronounced fibrosis and fatty degeneration as in toxic cirrhosis of the liver. After three months considerable improvement. Beginning of metabolic study one month after admission.

Case E

Man aged 56. Admitted to hospital and operated, cholecystectomy + choledocholithotomy. An T-tube was inserted. Postoperative cholangiography revealed several calculi in the bile ducts and the liver tests remained pathological. Reoperation after six weeks. The patient made a good recovery. Beginning of metabolic study 12 days after first operation.

Case F

Man aged 58. Symptoms from the biliary tract for 11 years. After one month of increasing symptoms of cholelithiasis with jaundice was admitted to hospital for operation. Long and technically difficult operation due to pronounced cholecystitis. Postoperative course at first satisfactory with ordinary secretion of bile from the drainage tube and sufficient volume of urine. Three days after operation hepato-renal syndrome developed. The patient was transferred to the intensive therapy ward, where urine secretion soon started again. Due to cellulitis over half the area of the trunk septic picture developed and the

Albumin (g/100 ml)	Globulin (g/100 ml)	Prothrombin time (normotest) (Sample/Normal 10^2)	Creatinine (mg/100 ml)	Cholic acid clearance (T 1/2 min)
31	6 0	69	2.7	35
27	4 8	28	1.0	90
33	3.3	78	1 0	21
33	3 3	48	0.7	12
33	3 5	>100	1.2	11
37	4 6	28	2.7	27
26	2 4	14	3 1	
41	3 6	47	1.5	30
43	2.8	74	0.7	35
		32	0.8	77
1.6-4 6	2 0–3.5	>80	<1 4	12.6±1 6

patient lapsed into coma. He was placed in respirator was treated with neomycin via duodenal tube and with cloxacillinnatrium parenterally. Discharged in good condition two months after operation. Beginning of metabolic study eight days after operation and three days after beginning of antibiotic treatment.

Case G

Man aged 60. Admitted to hospital after less than one day' symptoms of increasing tiredness, intense nausea, vomiting, aching pain in the liver tract, diarrhea, fainting fits, icteria. Lower margin of the liver was palpable 5 cm below the right costal margin. At exploratory laparotomy the diagnosis became acute enteritis. Treated in respirator. A hepatic coma developed and, in spite of intense treatment including terramycin, the patient died eight days after operation. Autopsy revealed necrosis of about 2/3 of the liver (picture as in infectious hepatitis and some intoxication) No signs of cirrhosis or malignancy. Beginning of metabolic study one day after operation.

Case H

Man aged 55. Admitted to hospital two days after he had mistakenly swallowed draught of carbon tetrachloride. At first immediate gastro-intestinal reaction. No further symptoms for one day and then increasing nausea and pain in the right upper quadrant of the abdomen. Treated with parenteral fluid therapy Practically symptomfree after one week. Beginning of metabolic study four days after ingestion of carbon tetrachloride.

Case I

Man aged 70. Twelve years earlier operated for perforations of the gut caused by injury Now admitted to hospital for symptoms of malabsorption Liver palpable 10 cm below arcus. Liver biopsy revealed pronounced amyloidosis with no signs of underlying infection or malignancy The patient improved on substitution therapy discharged after 16 days. Beginning of metabolic study 11 da er admission.

Case K

Man aged 19 Admitted to hospital fo infectious hepatitis. Good improvement and discharged after four e Beginning of metabolic study nine days after first symptoms of hepatitis.

MATERIAL AND METHODS

Cholic acid 24-¹⁴C (New England Nuclear Corp Boston, Mass.) had specific activity of 40 μCi mg and purity was checked by thin-layer chromatography (TLC and autoradiography

Preparation and administration of labelled cholic acid Five μCi of cholic acid 24-¹⁴C as dissolved in ethanol and neutralized with NaOH After evaporation the material was sterilized by heating to 140°C for 3 hours and redissolved in 5 ml of sterile 1.4 % $NaHCO_3$ After injection serial blood specimens ere obtained from vein other than the one injected 20, 40, and 60 min after injection.

Urine as collected 0–4, 5–8 and 9–24 hours after injection and then daily for 4–10 days Toluene as used as preservative.

Determination of serum clearance of isotopic cholic acid. The total amount of ¹⁴C in serum as determined in duplicate by the oxygen flask combustion technique using aliquots of 0.2 ml serum. Counting as done in Packard Tri-Carb liquid scintillation spectrometer using the $^{14}CO_2$-absorption and scintillation solvent given by Davidson and Oliverio (3): phenethylamine 270 ml, methanol 270 ml, toluene 460 ml, PPO 5 g, POPOP 100 mg. The cpm in serum samples at 20, 40 and 60 min were semilogarithmically plotted against time. The half-life of injected cholic acid was determined graphically (1)

Fractionation of urinary labelled bile acids An aliquot of urine as acidified to pH 1 with hydrochloric acid and extracted with ethyl acetate followed by butanol Labelled unconjugated bile acids and glycine conjugates were recovered in ethyl acetate extracts, whereas labelled taurine conjugates appeared in the butanol extracts. Isotope was measured by the oxygen flask combustion technique and the total urinary isotope excretion was calculated as the sum of isotope in the ethyl acetate and butanol extracts, as no isotope was found in the remaining water phase.

The ethyl acetate extract was subjected to rev

phase partition chromatography on columns of hydrophobic Hyflo Super-Cel (Johns-Manville, New York): moving phase methanol/water 150:150 and stationary phase chloroform/isooctanol 15:15 ml (8). A 4.5 g column was used when the amount of material to be analysed was less than 50 mg. For larger amounts the columns were correspondingly increased. The fractions collected from the columns were titrated with methanolic sodium hydroxide and isotope was determined in aliquots by liquid scintillation technique.

The chromatographic fractions and original ethyl acetate and butanol extracts were separated by TLC using the phase systems described by Eneroth (4) and Gänshirt et al. (6). The labelled compounds were detected by autoradiography.

Fractionation of biliary labelled bile acids. Bile was fractionated in the same way as described for urine.

RESULTS

Initial serum clearance of cholic acid 24-¹⁴C. Table I gives the half life of intravenously injected cholic acid 24-¹⁴C in serum of nine patients. The half life of cholic acid determined by Blum and Spritz (1) in a control group was 1 6 _ 1 6 min (mean ± S.D.)

The cirrhotic patients with severely impaired h function (A B C) had prolonged half-life approximately 3 7 and 2 times the normal value respectively whereas the cirrhotic patient D without icterus had a normal half-life. Patients H, I and K also showed prolonged half-life.

Of the two patients with biliary drainage and icterus, F showed a prolonged half life whereas E had a normal half-life.

Urinary excretion of isotope 1–5 days after administration. The cumulative urinary excretions of injected isotope in the patients are shown in Figs. 1–3.

In patient C the urine collection during the second day was unsatisfactory due to the patient's mental confusion. In patient G the irregular excretion curve was due to periods of oliguria and anuria during the first two days after administration.

The patients with biliary drainage (E, F) had urinary excretions below %. In the cirrhotic patients (A–D) the isotope excretion during five days after administration varied between 3 and 8%. The values obtained for patients G, H and I were in the same range. The highest urinary loss of isotope was observed in patient K with infectious hepatitis: 12% of injected isotope was ex

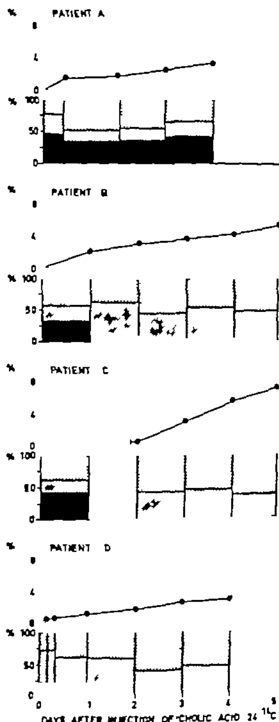


Fig. 1. Loss of isotope in urine after i. administration of cholic acid 24-¹⁴C to patients A–D and the composition of urinary labelled metabolites.

Curve: cumulative percentage urinary loss of i administered isotope. *Diagram:* percentage distribution of urinary labelled metabolites. ■ = Unconjugated bile acids. □ = Glycine conjugates. □ = Bile acid conjugates other than glycine conjugates.

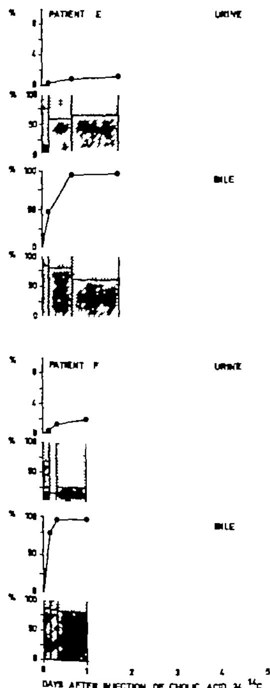


Fig. 2. Loss of isotope in urine and bile and the composition of urinary and biliary labelled metabolites. Patients E and F. For explanation see Fig. 1

Fig. 3. Loss of isotope in urine and the composition of urinary labelled metabolites. Patients G–K. For explanation see Fig. 1

creted in the urine during the four days after administration.

Biliary excretion of isotope. In patients E and F who both had external biliary drainage, almost all injected isotope was excreted in bile within 24 hours. Afterwards no isotope was detected in bil

Labelled urinary metabolites. Each urine s

Table II *Excretion of labelled bile acids as unconjugated bile acids glycine conjugates, other conjugates than glycine conjugates*

Pat.	Sampling period of urine (day) after injection of cholic acid 24-¹⁴C)	of given isotope excreted in urine			Percentage distribution of labelled conjugates recovered in	
		A conjugated labelled bile acids	As unconjugated labelled bile acids	Total	Glycine conjugates	Conjugates other than glycine conjugates
A	4	2.0	1 4	3.4	36	64
B	5	4 9	0 4	5.3	46	54
C	5	6.3	0.9	7..	37	63
D	4	2.8	0 2	3.0	46	54
E	2	1.2	0 04	1.2	58	42
F	1	1 9	0 08	2.0	22	78
G	3.5	3.3	1 4	4 7	63	37
H	5	6.3	0.2	6.5	58	42
I	4	4 4	0.2	4 6	27	73
K	4	12.0	0 3	12.3	23	77

men was fractionated by extraction and chromatography The percentages of glycine conjugates, conjugates other than glycine conjugates and unconjugated bile acids were determined and the results are summarized in Figs. 1–3 and Table II.

Excretion of unconjugated bile acids. The first 8-hour specimen from all patients contained unconjugated bile acids. After 24 hours no further excretion of unconjugated bile acids was observed in patients E F and K. The highest excretions of unconjugated bile acids were observed in patients A and G After 3 days 42 and 35% respectively of total urinary isotope was excreted as unconjugated bile acid In patients B C, D H and I the excretion of labelled unconjugated bile acids after 3 days was very low not exceeding 4% of the total excreted isotope.

Ratio of labelled glycine conjugates to other labelled conjugates The percentage distribution of daily urinary isotope between glycine conjugates and other conjugates was determined (Figs. 1–3) The conjugates other than glycine are mainly taurine conjugates, but other unidentified conjugates are included in this group. The ratio between glycine conjugates and other conjugates was about the same in different specimens from the same patient However in patient H, an increase of the ratio was observed during the investigation

The percentage distribution of total excreted labelled conjugates between glycine conjugates and other conjugates is given in Table II. In patients with chronic liver diseases (A, B C, D and I) a dominance of conjugates other than glycine conjugates was observed. In patients with acute liver diseases (E, F G H and K) varying ratios between glycine conjugates and other conjugates were observed.

Labelled biliary metabolites. Bile obtained from patients E and F was analysed for labelled bile acids. The results are shown in Fig. 2 and indicate that no unconjugated bile acid was present in bile The labelled conjugates had the chromatographic behaviour of glycocholic and taurocholic acid.

The ratio of glycine conjugates to other conjugates was higher in bile than in urine.

DISCUSSION

Serum clearance of cholic acid 24-¹⁴C The present study confirms the results of other investigators (1 11) of a very slow disappearance from serum of isotopic cholic acid administered i.v. to patients with advanced Laennec's cirrhosis or viral hepatitis.

In addition we have found the same slow disappearance of injected isotope in patients with

other hepatobiliary diseases such as biliary obstruction due to cholelithiasis complicated with hepato-renal syndrome carbon tetrachloride liver injury and chronic liver disease without jaundice due to amyloidosis.

Urinary loss of isotope The mechanism of renal excretion of bile acids is not known in man. Investigations in dog (13) have demonstrated glomerular filtration and active reabsorption in the proximal tubule for taurocholate, glycocholate and cholate. An additional minor reabsorption of cholate in distal tubule was suggested. Thus in the dog the renal excretion of bile acids is limited by two factors:

1) limited glomerular filtration due to extensive proteinbinding and 2) active reabsorption of bile salts by the proximal renal tubule.

Similar renal excretion mechanisms may be also important in man. In patients with abnormally increased amounts of bile acids in serum, exceeding the bile acid binding capacity of the serum proteins, an increased glomerular filtration of bile acids would be expected. If in addition, proximal tubular active reabsorptive capacity is over whelmed, then an increased amount of bile acids would be excreted in the urine. In none of the adult patients studied, however does this seem to be the case. Common to all the patients studied is that only a small fraction of the cholic acid pool is eliminated by urinary excretion. Even patient G with massive hepatic necrosis, who died a week after the beginning of the study eliminated only 5% of the isotope given by urinary excretion during the four days following the administration.

These results obtained in adults with liver disease are in striking contrast to the results obtained in infants with different liver diseases. Infants with neonatal hepatitis syndrome (9) excreted almost all of the injected isotopic cholic acid in urine and only trace amounts were recovered in feces.

Ratio unconjugated to conjugated bile acids in urine and bile The activity of the conjugating enzyme system seems to be reduced to only a rather small extent in these patients with different hepatobiliary diseases. In only two of the patients was constant urinary excretion of labelled unconjugated bile acids demonstrated. The two patients with obstructive jaundice and biliary drainage excreted almost all isotope in bile within 24 hours. All isotope in bile and almost all of the small amounts of isotope e[illegible]eted [illegible]n urine were in the conjugated form [illegible]nd [illegible] th[illegible]t in these patients, one with rather he[illegible] [illegible]red liver function the liver cells still h[illegible] [illegible] to conjugate and excrete bile [illegible] [illegible]pletely

Ratio labelled glycine con[illegible] [illegible]jugates than glycine conjugate [illegible] bile the ratio between glycine and t[illegible]jugates is around 3 1 In chroni[illegible] decrease of the ratio to or below [illegible] observed (10) The excretion [illegible] [illegible]cine conjugates in urine from pa[illegible]rhosis, acute hepatitis and bil[illegible] [illegible] found to be about 1 1 (5) In the [illegible] tigation we found that the ratio of l[illegible]lled [illegible] conjugates to other conjugates in uri[illegible] but was always below 1 1 in patients w[illegible] chronic liver disease.

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MUSCLE AND LIVER SERUM ENZYME ACTIVITIES IN HEALTHY VOLUNTEERS GIVEN ALCOHOL ON A DIET POOR IN CARBOHYDRATES

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Abstract. In order to elucidate the importance of a shortage of carbohydrates in the food for the occurrence of alcoholic muscle disease, muscle and liver enzymes (LDH isoenzymes, GOT, GPT and CPK) have been assayed in the serum of six healthy volunteers after alcohol ingestion on a diet poor in carbohydrates (group I). Twelve healthy subjects fed the carbohydrate-poor diet without alcohol addition (group II) and six other subjects given alcohol when on the average Swedish diet (group III) served as controls. In the group given alcohol in addition to the carbohydrate-poor diet an increase of LDH-1 and LDH-2 was observed. No other noticeable enzyme increases were registered. It is concluded that a lack of carbohydrate may be a factor involved in the pathogenesis of alcoholic myopathy.

Numerous attempts have been made to discover whether disorders associated with alcoholism are due to nutritional deficiencies alone or to the combined effect of alcohol and an inappropriate diet. These studies have mainly concerned the pathogenesis of alcoholic liver disease (15).

The pathogenesis of alcoholic myopathy, however, has scarcely been studied. It has been supposed that a disturbance of the lactic acid production under conditions of ischemic exercise is involved in the pathogenesis of alcoholic myopathy (19). This seems unlikely, considering that a low lactic acid response to ischemic work occurs in patients with other acute disorders as well (17).

In an earlier study (4) an increase of the muscle-specific creatine phosphokinase (CPK) was noted when alcoholics were given alcohol with carbohydrate-poor diets, but not when fed normal hospital diets.

The purpose of the present investigation was to study the effect of alcohol and carbohydrate-poor diet upon muscle and liver enzymes in the serum of healthy volunteers.

PROCEDURES AND SUBJECTS

Alcohol administration. The subjects received 1 000 ml of 40% alcohol over a weekend according to the following schedule: Friday afternoon 200 ml divided into two doses, Saturday 400 ml and Sunday 400 ml divided into four doses.

Carbohydrate-poor diet. The diet consisted for example of lamb, pork, chicken, eggs, margarine, tomatoes, cod, herring, and was given for four days. The total calorie content of the diet varied from 1 500–2 000 kcal per day. When alcohol was added, the alcohol ingestion began in the afternoon of the second day and continued during the third and fourth day of the diet period.

The experimental subjects were 17 male and 7 female healthy medical students (aged 23–25) performing their basic training in internal medicine and surgery. Each subject participated only in one experiment. Before every experiment all the subjects displayed enzyme activities within the normal range of this laboratory.

Six subjects (group I) were given alcohol in addition to the carbohydrate-poor diet. A new carbohydrate-poor diet period without alcohol consumption was instituted on the 13th to the 16th day after the initial alcohol and diet period.

Twelve subjects (group II) were given the carbohydrate-poor diet without alcohol ingestion. Six of these twelve subjects were again fed the carbohydrate-poor diet on the 13th to the 16th day after the first diet period.

Six subjects (group III) were given alcohol when fed the average Swedish diet (10). The total calorie content was about the same as in the carbohydrate-poor diet.

Blood samples for enzyme analyses were drawn in the morning on the 1st, 3rd, 5th, 8th, 10th, 12th, 17th, 19th, 22nd, 24th and 26th days after the termination of alcohol administration and of the first diet period.

METHODS

All blood samples for enzyme analyses were drawn in the morning. Creatine phosphokinase (CPK), transaminases (GOT and GPT) and lactate dehydrogenase (LDH) were assayed on the day on which the sample was drawn. Determinations of the LDH isoenzymes were usually per-

Table I. *Mean enzyme increase above initial level*

Second line in group II from day 17 to 26 refers to the six subjects given the carbohydrate-poor diet again on days 13 to 16

	Days after the end of alcohol administration and/or diet period										
	1	3	5	8	10	12	17	19	22	24	26
LDH 1 U/ml											
Group I											
Mean	−1	4	12	26	32	19	20	42	42	18	27
S.E.M. (n = 6)	±10	±8	±9	±11	±8	±5	±7	±5	±14	±8	±18
Group II											
Mean	−2	7	5	3	3	10	16	3	−6	2	13
S.E.M. (= 12)	±5	±4	±4	±4	±5	±4	±7	±5	±2	±7	±14
Mean							−5	−6	−13	3	12
S.E.M. (n = 6)							±6	±6	±8	±6	±12
Group III											
Mean	−3	2	9	14	8	7	23	13	9	13	18
S.E.M. (n = 6)	±5	±1	±2	±7	±7	±8	±5	±7	±8	±9	±8
LDH 2 U/ml											
Group I											
Mean	−10	5	3	13	12	23	3	27	56	44	42
S.E.M. (n = 6)	±5	±3	±4	±10	±11	±10	±6	±9	±8	±14	±7
Group II											
Mean	5	8	10	8	14	7	9	14	10	9	−18
S.E.M. (n = 12)	±6	±4	±5	±3	±5	±3	±7	±6	±5	±8	±3
Mean							−2	−4	−20	−15	−19
S.E.M. (= 6)							±4	±7	±5	±6	±3
Group III											
Mean	14	5	−3	7	2	8	6	6	18	6	3
S.E.M. (n = 6)	±4	±5	±3	±5	±4	±5	±4	±4	±6	±5	±3
LDH 3 U/ml											
Group I											
Mean	−6	−2	−1	−6	−6	−1	−3	0	−3	−4	−1
S.E.M. (= 6)	±2	±1	±2	±5	±5	±3	±2	±3	±2	±1	±2
Group II											
Mean	−1	2	2	−1	0	−1	1	2	1	4	−3
S.E.M. (= 12)	±3	±3	±1	±2	±2	±3	±2	±2	±2	±4	±1
Mean							−4	−9	−5	−6	−6
S.E.M. (n = 6)							±1	±2	±5	±3	±3
Group III											
Mean	5	2	2	10	8	7	7	8	6	1	7
S.E.M. (n = 6)	±2	±2	±2	±2	±3	±5	±2	±3	±3	±3	±3
LDH-4 U/ml											
Group I											
Mean	−2	0	1	−3	1	5	0	3	4	1	2
S.E.M. (= 6)	±1	±1	±1	±2	±2	±1	±1	±1	±2	±3	±1

Table I (*continued*)

	Days after the end of alcohol administration and/or diet period										
	1	3	5	8	10	12	17	19	22	4	6
Group II											
Mean	1	1	1	0	1	0	3	1	0	3	
S.E.M. (n = 12)	±1	±1	±1	±1	±1	±1	±2	±1	±2	±2	±1
Mean							−2	−5	−2	−3	−
S.E.M. (n = 6)							±1	±1	±1	±1	±1
Group III											
Mean	2	0	0	3	1	2	2	2	1	−1	1
S.E.M. (n = 6)	±2	±1	±1	±1	±1	±2	±1	±1	±1	±2	±
LDH-5 U/ml											
Group I											
Mean	−1	1	0	−2	2	2	0	3	4	1	2
S.E.M. (n = 6)	±1	±1	±1	±1	±2	±1	±1	±1	±1	±1	±1
Group II											
Mean	0	1	2	0	1	0	1	1	0	1	−2
S.E.M. (n = 12)	±1	±1	±1	±1	±1	±1	±2	±1	±2	±2	±1
Mean							−3	−3	−1	−2	−2
S.E.M. (n = 6)							±1	±1	±1	±1	±1
Group III											
Mean	2	2	2	1	0	1	0	−1	−1	−2	−1
S.E.M. (n = 6)	±2	±1	±1	±1	±1	±1	±1	±1	±1	±2	±2
GOT U/ml											
Group I											
Mean	8	6	9	4	5	5	4	7	7	6	6
S.E.M. (n = 6)	±2	±3	±3	±2	±2	±2	±4	±3	±3	±3	±3
Group II											
Mean	3	3	1	0	1	1	3	4	−6	−1	2
S.E.M. (n = 12)	±3	±3	±2	±2	±2	±3	±4	±3	±2	±4	±3
Mean							12	16	9	7	9
S.E.M. (n = 6)							±3	±3	±2	±3	±2
Group III											
Mean	2	3	4	−2	1	8	11	10	6	9	12
S.E.M. (n = 6)	±3	±2	±2	±3	±3	±6	±3	±2	±3	±2	±1
GPT U/ml											
Group I											
Mean	8	6	9	4	5	5	4	7	7	7	6
S.E.M. (n = 6)	±2	±3	±3	±2	±2	±2	±4	±3	±3	±3	±3
Group II											
Mean	7	4	7	3	4	4	5	0	−2	−3	4
S.E.M. (n = 12)	±2	±2	±2	±2	±2	±2	±2	±2	±1	±2	±2
Mean							6	−1	1	1	−1
S.E.M. (n = 6)							±3	±2	±2	±3	

Table I (*continued*)

	Days after the end of alcohol administration and/or diet period										
	1	3	5	8	10	12	17	19	22	24	26
Group III											
Mean	2	3	6	−1	0	0	5	4	−5	−2	1
S.E.M	±2	±2	±2	±1	±1	±2	±3	±3	±3	±3	±2
(=6)											
CPK U/ml											
Group I											
Mean	1	0	1	0	−1	3	0	1	2	0	−1
S.E.M.	±1	±1	±2	±3	±2	±2	±2	±2	±2	±1	±1
(=6)											
Group II											
Mean	0	1	−2	−1	−2	2	0	2	2	4	2
S.E.M.	±1	±2	±1	±1	±1	±2	±1	±3	±2	±1	±1
(n=12)											
Mean							−2	−2	−3	−1	−2
S.E.M							±2	±1	±1	±1	±1
(=6)											
Group III											
Mean	4	2	2	2	0	5	0	3	2	3	5
S.E.M.	±2	±2	±3	±2	±2	±3	±1	±2	±2	±3	±3
(=6)											

formed on the day on which the sample was drawn. Otherwise the samples were stored t room temperature for at most ..4 hours.

CPK was determined as described I Sigma Technical Bulletin, no. 661 (20).

Table II *The mean LDH 1 and LDH 2 increase above initial level before and after the second diet period (see text)*

	Before second diet period		After second diet period	
	LDH 1	LDH-2	LDH 1	LDH 2
Group I				
Mean	12	9	24[a]	31[b]
S.E.M	±7	±6	±5	±11
(n=6)				
Group II				
Mean	4	10	1	9
S.E.M	±3	±3	±6	±6
(n=12)			(=6)	
Mea			5	−8
S.E.M.			±5	±4
(=6)				
Group III				
Mean	6	5	15	7
S.E.M.	±3	±3	±4	±2
(=6)				

Significantly different from group II ($p < 0.01$).
[b] Significantly different from groups II and III ($p < 0.05$).

GOT and GPT were assayed by a kinetic UV method using reagents from Kabi, Stockholm, Sweden (12).

LDH was measured by an automatic fluorimetric technique (6).

Determination of LDH isoenzymes was done by electrophoresis on agar gel (21).

RESULTS

LDH isoenzymes An increase of LDH 1 and *LDH 2 was observed during the last week of the* sampling period in the subjects given alcohol when fed the carbohydrate-poor diet (group I, Table I).

For each subject the mean was calculated for all the enzyme increases or decreases obtained before the second diet period (e.g. days 1 3 5 8, 10 and 12) Similar individual means were determined from the enzyme changes after the second diet period (e.g. days 17 19 22, 24 and 6).

This yields six observations per group before and after the second diet period, which serve as basic data in testing the significance of group differences (Table II) F-tests revealed significant differences between the group means for LDH 1 and LDH-2 after the second diet period ($p < 0.01$). Following the significant results of the F-test, Student's *t*-tests indicated significant differences

between LDH 1 in group I and LDH 1 in group II ($p < 0.01$). In the same way the mean for LDH-2 in group I after the second diet period was significantly different from the means for groups II and III ($p < 0.05$)

When the mean increments on a specific day were tested by F-tests, significant differences were found on days 19 and 22 for LDH 1 and on days 22, 24 and 26 for LDH 2 ($p < 0.01$). Student's *t*-test implied that the mean increases of LDH 1 and LDH 2 in group I on these days were significantly higher ($p < 0.01$) than in the other groups.

GOT GPT and CPK were practically unchanged during the whole period of observation in all three groups.

DISCUSSION

There are some observations showing that a carbohydrate-poor diet may have an unfavourable effect upon skeletal muscle. By administering a low carbohydrate diet Hed (9) could provoke an attack in three brothers with familial myoglobinuria. Engel et al. (5) showed in a pair of identical twins that fasting or high-fat low-carbohydrate diet provoked cramping muscle aches and a marked rise of serum GOT GPT CPK and LDH. Creatinuria, which is a feature of diseases of the muscle, is related to carbohydrate metabolism. Thus starvation results in creatinuria, which disappears after administration of carbohydrate but not after administration of fats or protein (3). Brentano showed that the urinary output of creatine inversely followed the glycogen content of the muscles (1 2) As regards skeletal muscle glycogen, Hultman has shown that the resynthesis of glycogen is delayed unless carbohydrates are included in the food (11).

It is now firmly established that ethanol can inhibit hepatic glyconeogenesis when consumed in the fasting or semi-fasting state (7 8 16). There is also evidence indicating that alcohol can block gluconeogenesis when given with a low-carbohydrate high-fat diet (14). Moreover utilizing isotopic techniques for quantification of the lactate and glucose interconversion, it was found that starvation might not be a prerequisite for ethanol inhibition of gluconeogenesis in humans (13) Thus alcohol consumption on a carbohydrate-poor diet (group I) might have resulted in a subliminal glucose supply to the periphery In that respect the state in group I is similar to that prevailing in alcoholics, since alcoholics usually eat erratically if anything, during an alcoholic bout.

In this investigation with healthy volunteers an evident increase of LDH 1 and LDH 2 occurred when alcohol was consumed in addition to the carbohydrate-poor diet. An LDH 1 and LDH 2 increase has earlier been reported in alcoholics and interpreted as due to a muscle tissue damage (18). The alcohol consumption with a normal diet or the carbohydrate poor diet alone without alcohol addition did not cause any noticeable increments of the assayed serum enzymes.

To conclude, a lack of carbohydrates may be a factor involved in the pathogenesis of alcoholic *muscle disease*

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Table I *Thrombocytopenia in one hospital region of Sweden (1.2 mill. inhabitants)*

Year	Diagnoses registered	Incorrect diagnoses	No. of admissions of thrombocytopenias	No. of individuals	Drug-induced thrombocytopenia (not previously registered)[a]	No of pats. with thrombocytopenia	Patient records Missing	Patient records Available
1964	106	5	101	82	—[b]	82	5	77
1965	80	1	79	64	—[b]	64	11	53
1966	93	4	84	61		63	4	59
1967	114	4	110	65	3	68	7	61
1968	132	—	132	80	2	82	7	75
	525	*14*	*511*	*352*	*7*	*359*	*34*	*325*
			139 (31 %)				9[a]	

[a] Patients reported to the Swedish Adverse Drug Reaction Committee but not found in the data lists.
[b] Adverse drug reactions not reported until late in 1965

(Table IV) During the years of childhood and up to puberty there are as many cases among boys and girls, at all other ages more than twice as many women as men but varying in different age groups (Table V)

The causes of thrombocytopenia and of the neonatal cases are shown in Table VI. In 152 cases (47 %) no definite cause of thrombocytopenia could be found, either primarily or in the later perusal of the medical records, and the condition was therefore classified as idiopathic or essential. As regards the other diagnoses, only in a small number of cases could the cause be confirmed on the basis of provocation or course—for the most part the causes must be denoted as "probable

The various groupings of causes differ in age and sex distribution (Table VII). The cases with infectious cause are younger than the average, the drug cases older The cases with essential thrombocytopenia are younger than those with known causes.

The platelet counts are presented in Fig. 2, which shows the lowest initial count for each case The counts have a skew distribution, the median is 23 000 There is no difference between different diagnosis groups (Table VII), nor between men and women.

The treatment will be seen from Table VIII. Steroid therapy was given to 139 patients (43 %),

Table II. *Missing patient records*

Age group	Men	Women	Total
0– 4	2	1	3
5–14	3	3	6
15–24		1	1
25–34		1	1
		1	1
45–54		5	5
55–64	1	2	3
65–69	1	1	2
70–74	1	2	3
> 75	4	5	9
	12	*22*	*34*

Table III. *Age and sex distribution of patients with thrombocytopenia (total 5-year material)*

Age group	Men	Women	Total
0– 4	22	8	30
5–14	22	21	43
15–24	14	18	32
25–34	4	15	19
35–44	6	25	31
45–54	10	22	32
55–64	15	38	53
65–69	9	22	31
> 70	36	52	88
	138	*221*	*359*

Table IV *Thrombocytopenia in childhood*

	♂	
Newborn	8	1
Others 1 y	7	
Total 1 y	15	1
2–4 y	5	6
5–10 y	12	10
Total 10 y	17	16



Fig. 1 Incidence of thrombocytopenia in various age groups. — — = men, + + = women, O—O = total.

83 of whom with good and 14 with doubtful effect. In 42 of the 139 steroid-treated cases (30 %) no effect was obtained on the platelet count. Altogether 64 cases (19 %) had been splenectomized, of which 50 after initial steroid therapy.

Altogether 15 patients (5 %) died as a result of thrombocytopenia with different forms of haemorrhage: cerebral (6 cases), intestinal (6 cases), pulmonary (2 cases) and in conjunction with childbirth (1 case). The mean age of fatal cases was 62 years, the platelet counts being low: mean 17 000.

An attempt has been made to estimate the number of chronic cases (Table IX). The classification is based on the primary data in the medical records and is entirely dependent on their completeness. It will be seen that the number of cases with acute course is considerably higher in the group "others" than among the essential thrombocytopenias.

Table V. *Sex distribution*

Age group	Women/men
0– 1	0.07
2– 4	1.00
5–14	1.00
15–24	1.29
25–34	3.75
35–44	4.17
45–54	2.20
55–64	2.53
65–69	2.44
70	1.44
Total	1.60

Table VI. *Causes of thrombocytopenia*

Essential	152	47	
Drug-induced (probable)	42	13	18
Drug-induced (possible)	16	5	
Acute infection	34	10	
Heart disease	28	9	
Malignant neoplasm	11		
Collagen disease	10		
Hepatic cirrhosis	10		
Neonatal	10		
Haemolytic anaemia	6		
Diabetes mellitus	5		
Intoxication (tear gas)	1		
	325		
Neonatal thrombocytopenia			
Essential thrombocytopenia in the mother	3		
With congenital malformations	2		
Rubella embryopathy	1		
Unknown cause	3		
	9		

Table VII. *Age and sex distribution in different diagnostic groups*

			Mean age (y.)	No. of platelets (mean value)
Essential thrombocytopenia				
Men	62	41	38	30 000
Women	90	59	41	29 000
	152		*40* ($p < 0.001$)	*30 000*
Drug-induced thrombocytopenia				
Men	14	23	50	30 000
Women	44	77 ($p < 0.001$)	58	25 000
	58		*59* ($p < 0.001$)	*26 000*
Infection-induced thrombocytopenia				
Men	15	44	21	21 000
Women	19	56	36	42 000
	34		*29* ($p < 0.001$)	*33 000*
Total				
Men	130	40	42	30 000
Women	195	60	49	31 000
	325		*46*	*30 000*

Deviations from the mean regarding age and/or sex distribution has been analysed by the χ^2-test. Results are indicated by p-values.

Table VIII. *Treatment of thrombocytopenia*

		Later splenectomy	Died of thrombocytopenia	Platelet values (mean)
Essential thrombocytopenia				
Steroid therapy				
Good effect	51	15		
Doubtful effect	8	5		
No effect	29	18	4	
	88			20 000
No steroid therapy	64	10	3	44 000
	152			
Drug-induced thrombocytopenia				
Steroid therapy				
Good effect	9	1		
Doubtful effect	1	1		
No effect	5	1	1	
	15			16 000
No steroid therapy	43		3	33 000
	58			
Other thrombocytopenia				
Steroid therapy				
Good effect	23	3		
Doubtful effect	5	2		
No effect	8	4		
	36			26 000
No steroid therapy	79	4	4	38 000
	115			
All patients				
Steroid therapy				
Good effect	83	19		
Doubtful effect	14	8		
No effect	42	23	5	
	139 (43 %)	*50*	*5*	
No steroid therapy	*186*	*14*	*10*	
	325	*64*	*15*	
		(19 %)	(5 %)	

DISCUSSION

Idiopathic thrombocytopenic purpura (ITP) is, by definition, the designation for cases of thrombocytopenic purpura without known aetiology. The name has been used very differently, however, and in several recent surveys one finds among ITP cases large groups with definite or probable aetiology (9, 10, 13). This makes it difficult to compare different materials. The same applies to the concept "purpura" which forms part of the name of the disease but is usually not required for diagnosis.

It has been maintained (1) that the essential causative factor of purpura is an increased capillary fragility, but that the haemorrhagic tendency

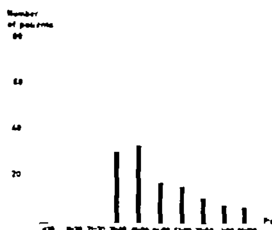


Fig. 2. Distribution of initial platelet values. Up-to-d… values are missing for 6 patients with chronic throm… cytopenia.

may be increased through the simultaneous e… istence of thrombocytopenia and that the thro… bocytopenia is probably caused by the sa… factor which damages the vascular wall—the… are antigenic similarities between platelets a… vascular endothelium.

Karpatkin (8) has thoroughly discussed the problems in an admirable recent survey as al… the artificial division into different forms… course (vide infra). He describes how by differe… techniques, one can now demonstrate antibodies… platelets (of IgG type) in 65% of the cases call… idiopathic and suggests the name autoimmu… thrombocytopenic purpura for these cases, whi… reserving the term idiopathic for the 35% … which antibodies are not demonstrable.

The material analysed here consists of cas… which for one reason or another have been foun… to have a platelet count below 100 000/mm³ wi… or without purpura, and irrespective of cause.

The number of cases of such thrombocytopeni… is fairly constant from year to year averaging 7… cases per 1.2 mill. inhabitants and annum in th… Uppsala region. For the whole of Sweden (8 mill.

Table IX. *Course of thrombocytopenia*

Thrombocytopenia	Acute	Chronic	Dead	Evaluation not possible	Tot…
Essential	37 (24 %)	94	9	12	152
Other	85 (49 %)	52	16	20	173
	122	*146*	*25*	*32*	*325*

this would amount to about 480 cases per annum. The incidence differs greatly for men and women—on an average there are twice as many women as men among thrombocytopenia cases. Excluding children under 14 years the incidence is 4.2 males to 8.1 females per 100 000 inhabitants and year

The reason for this sex difference is not known. A remarkable deviation occurs at the very youngest ages at which, in contradistinction to other ages, male patients predominate. This may be a chance occurrence in a fairly small material, but the male preponderance below 1 year of age is nevertheless marked (Table IV) Up to puberty there are then as many girls as boys. The male preponderance has not been observed in other reports (2, 9 13) though a uniform sex distribution prior to puberty has been reported (12)

The large number of cases at high ages is remarkable. It has earlier been reported that most cases of thrombocytopenia occur between 5 and 30 years of age and that it is less common after 30 years of age (14) Wintrobe (15) surveying his own and others cases, found 64 % to be younger than 21 years. Meshaka et al. (10) found only 11 % of their 147 patients to be above 50 years of age. In our material 54 % of patients were above 50 years. The age shift is still more marked if the material is divided up according to aetiology Among those classified as essential thrombocytopenia 39 % were above 50 years, in the drug-induced group, on the other hand, no less than 77 % (the difference is significant, $p <$ 0.001) This change in age composition must be due to a change of the causes of thrombocytopenia, with a heavy increase in the number of drug-induced cases (3)

The mean age differs for cases with different causes (Table VII). The infectious cases are considerably younger than the average, the drug-induced cases older Cases with essential thrombocytopenia are younger than those with all known causes. The proportion of women is significantly higher in the drug-induced group than in the remainder of the material.

As appears from Table VI, the main group of cases (47 %) is described as essential thrombocytopenia. Despite a greater knowledge of several conceivable mechanisms—autoimmune sensitization to external agent (e.g. drug) toxic bone marrow injury infectious aetiology etc.—there remains this large group of unknown aetiology

Among known causes drugs predominate In 44 cases of thrombocytopenia (13 %) drugs were in all probability the cause In another 16 cases a possible cause. Drugs were responsible for 18 of all cases of thrombocytopenia The present drug-induced cases, together with the material of the Adverse Drug Reaction Committee will be the subject of a separate article (3) Here it may be stated solely that the largest groups are oral diuretics (thiazides, chlorthalidone furosemide) and quinine/quinidine drugs

In 34 cases (10 %) it has been considered that infections might have been the cause of thrombocytopenia. Grøttum (7) has pointed out that virus infections are not seldom accompanied by thrombocytopenia. Especially in children virus infections would seem to be an important aetiological factor Lusher and Zuelzer (9) found in 122 (84 %) of 146 cases in children below 12 years that, within 3 weeks before the onset of thrombocytopenia, the patients had an acute febrile disease probably of virus type one fifth of whom with exanthema. Szeto et al. (13) found infection in 89 of 203 cases (44 %) the majority being children below 10 years of age.

Heart diseases (ischaemic heart disease hypertension, organic heart defects) have been combined into a separate group. This is justified by the fact that, among these diseases, there is in some cases a stasis-conditioned enlargement of the spleen which may contribute to or induce thrombocytopenia, and that practically without exception the patients had taken one or more potentially thrombocytopenia-inducing drugs, particularly diuretics. The latter applies also to the small group of diabetics—both insulin and oral antidiabetics are drugs which may cause thrombocytopenia. The collagen group comprises two types of cases, disseminated lupus erythematosus and advanced cases of rheumatoid arthritis with enlargement of the spleen (Felty's syndrome) The simultaneous occurrence of haemolytic anaemia, with or without splenomegaly and thrombocytopenia has been reported (16) and thrombocytopenia in hepatic cirrhosis is well known.

The cause of three of the neonatal cases (Table VI) was that the mother had for a long time had sustained essential thrombocytopenia. In all of these three cases the child made a quick and spontaneous recovery

The platelet counts have a skew distribution

with a predominance of low levels, 47% of patients having counts below 20 000, 82% below 50 000 platelets/mm³. The median is 23 000. As appears from Table VII, there are no differences in platelet counts among different diagnosis groups, nor between men and women. On the other hand, the cases with the lowest platelet counts are found to be subjects to steroid treatment (Table VIII). The platelet counts are significantly lower in steroid-treated groups than in those which had not received steroids.

It is often pointed out that thrombocytopenia occurs in an acute and in a chronic form, sometimes mention is made of a subacute (10) and of a periodic form (4, 5). No sharp borderline can be drawn between these different forms, any more than between essential thrombocytopenia and thrombocytopenia of known aetiology. An attempt has, however, been made in the present material to assess the type from the medical records. It is found that roughly equal numbers had an acute and a chronic course. But there is a significantly greater proportion of acute cases in the group with known cause than in that with essential thrombocytopenia.

Most of the 15 deaths occurred in the non-steroid-treated groups. Apart from haemorrhage, most cases had other contributory causes of death —high age, ischaemic heart disease, earlier operated tumour etc. A 1 year-old boy died of cerebral haemorrhage directly related to his thrombocytopenia. A 31-year-old woman died after childbirth in serious haemorrhage. Owing to incomplete medical records it cannot be decided whether she had a primary thrombocytopenia or whether the original haemorrhage had another cause and the thrombocytopenia was secondary as a result of consumption in conjunction with heavy blood transfusions. It should be pointed out that another 13 deaths occurred in direct connection with the stay in hospital. They all died of other causes than thrombocytopenia and are not further discussed in this context.

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THROMBOCYTOPENIA

II. *Drug-induced Thrombocytopenia*

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Abstract. An analysis of 126 cases of drug-induced thrombocytopenia shows that oral diuretics (52 cases) and quinine/quinidine drugs (26 cases) are the entirely predominant causes. Drug-induced thrombocytopenia has favourable prognosis, and remission occurs usually within 1–2 weeks; 70% of the cases are women. The incidence of thrombocytopenia after 30 years of age, both that which is definitely drug-induced and that due to other causes, is markedly parallel to the consumption of group of potential thrombocytopenia inducing drugs, oral diuretics. The risk of thrombocytopenia through the use of oral diuretics may be calculated at 1 case/15 000 users, equal for men and women, but with rising risk at higher ages.

Haematological side-effects play an important role among the complications induced by drugs. In the Swedish Adverse Drug Reaction Committee's register they constitute 10% of all reported cases. All types of bone marrow cells may be affected, all at once or one or the other cell line specifically. Drug-induced agranulocytosis in the Swedish adverse drug reaction material has recently been studied (21). This is an analysis of drug-induced thrombocytopenia comprising 126 cases from the period 1964–70.

MATERIAL AND METHODS

The Swedish Adverse Drug Reaction Committee received [illegible] reports of thrombocytopenia (Table I) during the first five years of its existence (1966–70). In 13 of these cases the relation with the drug was judged to be less probable. In a general survey of thrombocytopenia from all causes a further 31 cases were discovered which had not been reported to the Committee (6). There are accordingly altogether 126 cases of drug-induced thrombocytopenia for analysis in which the relation between drug and thrombocytopenia was judged by the Committee to be "probable". The assessment of the 31 unreported cases has been made on the principles followed by the Committee.

Both in the Committee's material and in the other, larger material there are a number of cases (13+16 cases) in which the relation between drug and complication is less probable though not out of the question. These 29 cases have been analysed separately.

RESULTS

The number of drug-induced thrombocytopenias since 1966 has been fairly constant from year to year 25–30 cases annually (Table I). The age and sex distribution is given in Table II and the incidence is presented in Fig. 1 which shows the number of cases of thrombocytopenia—both sexes—at different ages in relation to the population of Sweden (number of cases per 1 mill. inhabitants in the respective age groups). The increase in number of cases of thrombocytopenia is especially great after 40 years of age.

Fig. 2 shows the age related incidence of thrombocytopenia from all causes.

Fig. 3 shows the number of persons in a Swedish area using oral diuretics. The curve was obtained from data registration of drug sales per individual and covers a 16-month period during 1968–69.

Fig. 4 is a combination of Figs. 1–3 and shows the remarkable parallelism between the incidence curve for thrombocytopenia of all causes (above the age of 30) and that for drug-induced thrombocytopenia, as well as between those curves and that describing the use of oral diuretics.

The sex distribution exhibits a strong predominance for women, who constitute 72% of

Table I. *Drug-induced thrombocytopenia*

	Reported to the Committee		Not reported		Total		
Year	Probable	Less probable	Probable	Less probable	Probable	Less probable	Total
1964			6	2	6	2	8
1965			5	1	5	1	6
1966	13	2	4	3	17	5	22
1967	12	5	4	8	16	13	29
1968	21	1	12	2	33	3	36
1969	29	3			29	3	32
1970	20	2			20	2	22
	95	*13*	*31*	*16*	*126*	*29*	*155*

cases, i.e. a predominance of 2.6 : 1 over men (Table II). This ratio is higher than indicated for the total drug consumption (2), for which the male/female ratio was 1.3 : 1, but lower than for the agranulocytosis material, for which the ratio was 3.9 : 1 (21). These differences are, however, not statistically significant.

The thrombocytopenia-inducing drugs are shown in Table III. The number of drugs is higher than the number of patients, since in a small number of cases ($n = 8$) two equally "risky" drugs (e.g. thiazides and quinidine) were given simultaneously and induced thrombocytopenia. Both drugs were judged to be "responsible" in these cases. There are two groups of drugs in particular which predominate, viz. oral diuretics ~ 1 quinine/quinidine compounds. These two ., covering 52 and 26 cases respectively been analysed separately (Table IV). Quinine/quinidine is found to have a tendency to induce rather lower platelet counts than the diuretics, a difference which is probably significant ($p < 0.05$). This appears also to be reflected in the treatment. In the quinine/quinidine group no less than 81% were treated with steroids against only 25% in the diuretics group. The sex distribution in these two groups is even more displaced in the female direction than in the material as a whole. Among the quinine cases there are only 5 (19%) men, among the diuretic cases 13 (25%). The sex distribution for users of diuretics (Fig. 3) shows 27% men.

As appears from Table I, the relation between drug and thrombocytopenia was considered "less probable" in 29 cases. There are many factors contributing to this classification: complicated disease conditions, simultaneous administration of more than one drug, uncertain time relation, etc. An analysis of these 29 cases is shown in Table V.

Table II. *Patients with drug-induced thrombocytopenia*

Age group	Men	Women	Total
0–4		1	1
5–14	2		2
15–24		3	3
25–34	1	6	7
35–44	1	5	6
45–54	5	11	16
55–64	11	21	32
65–69	4	15	19
> 70	11	28	39
	33	*90*	*125**
	28%	72%	

* One report anonymous with regard to sex and age.



Fig. 1. Incidence of drug-induced thrombocytopenia (men and women together).



Fig. 2 Incidence of thrombocytopenia of all causes. From our previous paper (6). — —= men, + —+ = women, o—o = total.



Fig. 3 Incidence of the use of oral diuretics in the Östersund area (90 000 inhabitants). Symbols as in Fig. 2.

It is seen that in respect of age, sex distribution and platelet count the group does not differ from the larger group in which the correlation was considered "probable. The drugs used are also the same, apart from the fact that oral contraceptives occur more often in this uncertain material.

Thrombocytopenia usually appears suddenly and disappears quickly after withdrawal of the harmful agent. The post-treatment values are not always indicated often it is stated merely that they have returned to normal" The time data are also inexact, but normal" platelet counts occur strikingly often within 1–2 weeks after withdrawal of the harmful drugs.

Platelet counts (lowest initial count) in the entire material have a skew distribution as shown in Fig. 5 The median value is 22 000 (mean 26 000)

An attempt has been made to evaluate which patients have had symptoms of their thrombocytopenia and what were their platelet counts. All haemorrhagic symptoms, including petechiae, have

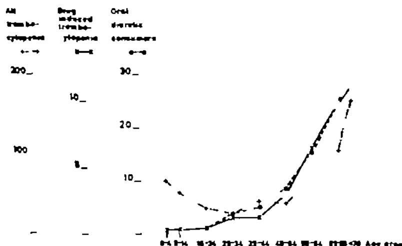


Fig. 4 Figs. 1–3 combined (in each case curve for total material). Note the close parallelism between the curves after the age of 30.

Table III. *Drugs causing thrombocytopenia*

Generic and Swedish registered names are given

Diuretics		52
Thiazides	21	
Chlortalidone	16	
Furosemide	12	
Clopamide	3	
Quinine/quinidine		24
Anti-phlogistics/anti-rheumatics		17
Phenylbutazone (Butazolidin®)	7	
Indomethacin (Indomee®)	6	
Oxyphenbutazone (Tanderil®)	6	
Metamizol (Buscopan® comp.)	1	
Acetyl salicylic acid	1	
Sulfonamides		11
Carbimazole (Neo-Mercazole)	4	
Sulfamethizole + *sulfamethoxi-* pyridazine (Sulfapral®)	2	
Salazosulfapyridine	1	
Chlorpropamide (Diabinese)	1	
Tolbutamide (Rastinon)	1	
Glymidine (Gondafon®)	1	
Unspecified	1	
Diphenylhydantoin (Difhydan®)		3
Nitrofurantoin (Furadantin®)		3
Oral contraceptives (Lyndiol® Follinyl®)		2
Antibiotics		3
Ampicillin		
V-penicillin		
Tetracycline		
Cytostatics		5
Busulfan		
Cyclophosphamide		
Azathioprine		
Thiotepa		
Melphalane		
Various drugs*		12
Hydralazine (Apresolin®)		
Ethosuximide (Suxinutin®)		
Phenprobamate (Gamaquil®)		
Acetazolamide (Diamox®)		
Dichlorphenamide (Daranide)		
Amitriptyline (Tryptizol®)		
Antazoline (Antasten®)		
Procainamide (Pronestyl®)		
Tikanox (combined sedative)		
"Polypharmacy"		
Paramethadione		
Phenbenzamine (Lergitin)		
		134

* One case each has been registered as caused by the drugs.

been recorded as positive symptoms. The results are shown in Table VI in which it is seen that most of the patients (68%) had symptoms of thrombocytopenia. Especially prominent is the haemorrhagic tendency at counts below 10 000 (89%), but it is high even up to 50 000 platelets/mm³ (66%) without any noticeable limit at any level between 10 000 and 50 000.

The diagnosis are listed in Table VII. The most prominent finding is that the "cardiac disease" group is so outstanding, amounting to 51%. The next group "joint-muscle diseases" amounts only to 14%.

Deaths in this material are few. Altogether 6 (5%) persons died *with* thrombocytopenia. Only 2 of them, however, died in haemorrhage directly related to a platelet deficiency, one after treatment of glaucoma with dichlophenamid (a 70-year-old man) and the other after treatment of joint symptoms with phenylbutazone (a 54-year old man). Two patients died in a picture of agranulocytosis and one in aplastic anaemia, none with bleeding symptoms. One patient died of heart failure with low platelet count after quinidine.

DISCUSSION

Side-effects of drug therapy are still reported to altogether too small an extent. For the 3-year period 1966–68 a comparison can be made in this material between the cases of thrombocytopenia reported and those discovered only through tracing of all cases of thrombocytopenia in a health service area. During the 3-year period 66 cases were reported from the whole of Sweden. During the same period 20 unreported cases of thrombocytopenia were discovered in the Uppsala region (1.2 mill. inhabitants). Calculated for the whole country (8 mill.) this would imply a probable occurrence of 133 *unreported* cases, i.e. a total of 199 cases of drug-induced thrombocytopenia of which *only* 66 (33%) had been *reported*. This figure may be compared with a reporting frequency of 22–40% (calculated on two Swedish areas) for a considerably more serious complication, namely agranulocytosis (21) and 20% for thromboembolism caused by oral contraceptives (5).

Drugs may induce thrombocytopenia in different ways: 1) *direct toxic effect*, usually on the megakaryocytes; 2) *immunological mechanism*, and possibly 3) through induction of *specific deficiency states*.

The toxic effect, e.g. of cytostatics, is probably rather uncommon as regards isolated thrombocytopenia. There is much to suggest that most cases have an immunological background. Harrington (10) was the first to prove by experiments on himself that thrombocytopenia could be in-

Table IV *Quinine- and diuretic-induced thrombocytopenia—a comparison*

The difference between the mean platelet values after quinine (18 000) and diuretics (29 000), respectively is probably significant ($p < 0.05$ χ^2-test)

	Initial			After therapy	
		Platelet value ($\bar{x} \pm s\bar{x}$)	Sex distribution (women/men)		Platelet value ($\bar{x} \pm s_{\bar{x}}$)
Quinine quinidine	26	18 000 ± 3 500	4.2:1	15	189 000 ± 19 000
Diuretics					
Thiazides	21	26 000 ± 3 400	4:1		
Chlortalidone	16	28 000 ± 4 400	2.2:1		
Furosemide	12	37 000 ± 7 800	1.4:1		
Clopamide	3	29 000 ± 18 000	2:1		
Diuretics total	52	29 000 ± 3 000	2.4:1	30	160 000 ± 18 000

duced by a humoral factor. Ackroyd (1) made clear the mechanism for the classical sedormid purpura, and it is now possible in a large number of cases to demonstrate antibodies to platelets, usually of type 7S-IgG (13, 20). Quite recently Eisner et al. (8) found antibodies of type IgM to platelets in thiazide-induced thrombocytopenia. Possibly this finding, together with the fact that the platelet counts in thiazide-induced thrombocytopenia appear to be rather higher than after quinine, may indicate a mechanism other than that in conjunction with quinine etc. Yunis (24) points to other possible mechanisms, e.g. due to a specific deficiency state which would be theoretically conceivable after treatment with folic acid antagonists, e.g. in the form of Septrin (vide infra)—in one case the platelet count became normal after administration of folinic acid (12).

Yunis states that agranulocytosis and aplastic anaemia—in that order—are the commonest drug-induced blood diseases. This is not in accordance with our results, which show that thrombocytopenia (25–30 cases annually) is more common than agranulocytosis (around 20 cases annually).

The prognosis for drug-induced thrombocytopenia is remarkably good. As noted, only in 2 cases did it lead to death in haemorrhage. The remission is also rapid in most cases, the platelet count returning to normal within 1–2 weeks. Kraytman (14) has recently shown that, in an

Table V *Less probable cases of thrombocytopenia*

29 (19 women, 10 men), mean age 50 y. Mean initial platelet value 23 000

Drugs used	No. of pats.
Oral contraceptives	5[a]
Sulfonamides	5
Oxyphenbutazone	3
Chlortalidone	2
Chloramphenicol	2
Diphenylhydantoin	2
V-penicillin	2
One case each after	
Phenylbutazone, Quinine, Indomethacin, Polythiazide, Methyldopa, Allopurinol, Ampicillin	7
Combined drugs	4
Total	32

[a] Oral contraceptives: Lyndiol® mite, Conluten, Delpregnin, Follutyl®, unknown.
[b] Sulfonamides: Sulfamethizol + sulfamethoxipyridazine (Sulfapral®), sulfamoxol (Sulfuno®), sulfametorin (Bayrena), unspecified (2 pats.).

Fig. 5. Distribution of initial platelet values in drug induced thrombocytopenia.

Table VI *The presence of haemorrhagic symptoms at various platelet levels*

	All patients	Patients with haemorrhagic symptoms	
	( )	(n)	(%)
< 10 000	35	31	89
10–49 000	67	44	66
> 50 000	18	7	39
	120[a]	82	68

Inadequate information in 6 cases.

experimentally induced thrombocytopenia in dog. the platelet count had returned to normal after only 4–5 days, which agrees with earlier reports of a platelet maturity time of around 4 days. He also showed that the platelet count rose more rapidly and to higher than normal levels, after repeated depletion of platelets.

The relation between drug prescription (cf the curve for consumption of diuretics in Fig. 3) and the prevalence of thrombocytopenia is striking. Thrombocytopenia *is stated in the literature to* occur chiefly in persons aged 5–20 years and to be uncommon after 50 years of age. The occurrence of thrombocytopenia from all causes (6) as would be expected, shows a predominance among young persons (Fig. 2) a reduced incidence in the thirties, and thereafter—contrary to earlier

le VII. *Occurring diagnoses*

	Men		Women		Total	
Heart disease		19		50		69
Valvular	1		4		5	
Ischemic	5		9		14	
Decompensation	7		9		16	
Hypertension	4		13		17	
Arrhythmias	2		15		17	
Joint-muscle disease		8		11		19
Infections		4		5		9
Diabetes		1		6		7
Epilepsy		3		2		5
Malignant disease				3		5
Thyreotoxicosis		2		1		3
"Nervosity		1		2		3
Restless legs				3		3
Various		1		12		13
		41		95		136[a]

Two major diagnoses given in 10 patients.

Table VIII *The calculated risk of getting diuretic-induced thrombocytopenia*

For all users of peroral diuretics	1:15 000
Age influence	
< 45 y	1:27 200
> 45 y	1:14 000
Sex influence	
Men	1:14 400
Women	1:15 100

reports—a rapid rise at higher ages to levels far above those occurring in the younger age groups. The drug induced thrombocytopenia group contains a significantly higher proportion of women *($p < 0.001$) and is significantly older ($p < 0.001$)* than the average for all thrombocytopenia cases. These circumstances, together with the close parallelism between the incidence curve for drug induced thrombocytopenia and for thrombocytopenia from all causes renders it highly probable that a very large number of thrombocytopenia cases above 40–50 years of age, which are now designated as essential are actually drug-induced.

Among individual groups of drugs the *oral diuretics* predominate There appears to be no difference between types of oral diuretics in their capacity to induce thrombocytopenia (Table IV). *This is also evident from an investigation by* Kutti et al. (15) who report an incidence of 27% cases of thrombocytopenia in chlorthalidone-treated cardiac patients and 26% in thiazide-treated. Contrary to earlier assertions, the thrombocytopenia after diuretics is practically as marked as after quinidine/quinine, which is the second largest group of thrombocytopenia-inducing drugs.

With a knowledge of the consumption of oral diuretics, as obtainable from the registration of drug sales per *individual, which is done by* data processing in the Östersund area (population 50 000) it is possible to calculate the risk of diuretic-induced thrombocytopenia (Table VIII). The overall risk is 1/15 000 users, practically equal for men and women. On the other hand, the risk increases greatly with rising age Somewhat more than 80% of the cases occur in patients above the age of 55 an age group constituting only 26% of the population.

The occurrence of thrombocytopenia *after* the use of oral diuretics has long been known and has been reported by several authors, also in Sweden

Table IX. *Reports on drug-induced thrombocytopenia, found in a Medlar (Karolinska Institutet) Search*

The table gives only the number of reports and not the number of patients. All reports on thrombocytopenia after chemotherapy of malignant disease have been excluded

Drug	No. of reports
Trimethoprim + sulfamethoxazole (Septrin, Bactrim)	6
Quinine	6
Oral diuretics	4
Chloramphenicol	4
Anti-epileptic drugs	3
Heparin	2
Oxyphenbutazone	2
"Sulfonamides"	2
Rifampicine	2
One case each after Methyldopa, amidopyrine, PAS, propranolol, paracetamol, acetyl salicylic acid, dextramene, penicillin, cephalosporin and gold salt	10

(15 19). The risk calculated here, and the apparently intimate connection between diuretics consumption and the occurrence of thrombocytopenia, appears to indicate the necessity for a greater vigilance in cases of thrombocytopenia—and perhaps a greater restrictiveness in the prescribing of diuretics. It is found that at the age of 20 approximately 1% of the population use oral diuretics, a figure that rises to 9% at the age of 40 and to 30% at the age of 70 (Östersund material).

In cases of hypersensitivity to quinine a single tablet, as also of sedormid, may bring on a serious thrombocytopenia (7). Whether the same applies to oral diuretics is not known today—as noted above, the mechanism may possibly differ from that of quinine.

In comparison with oral diuretics and quinine/quinidine, other drugs occur only sporadically with the exception of phenylbutazone/oxyphenbutazone and other antiphlogistics, which together account for a not insignificant number of cases of thrombocytopenia. Attention should also be directed to oral contraceptives and to the new compound daraprim (vide infra). Haemolytic anaemia following the use of methyldopa has long been known. It has now been proved that its use may give rise also to thrombocytopenia (16).

A survey of the "uncertain" cases (Table V) shows a picture entirely in accordance with that for the definite cases, suggesting a relation between the drug and thrombocytopenia also in the cases classified as "uncertain". There is a difference, however, namely no less than 5 women on oral contraceptives. It has been uncertain whether a relation existed in these cases. Here it may merely be pointed out that it has been proved (4) that oral contraceptives increase the sensitivity of platelets of adenosine phosphate (ADP) which can be measured by platelet electrophoresis. A surface-active factor, probably from lecithin, was considered responsible for this change. Quite recently Besterman and Gillett (3) demonstrated that lysolecithin can inhibit the platelet aggregation induced by ADP. These findings strongly suggest that oral contraceptives act upon the surface of the platelets and may cause their disintegration, leading to thrombocytopenia, perhaps through other mechanisms than those earlier discussed for sedormid and other drugs.

A rather more up-to-date picture of the drugs, which in recent years have induced thrombocytopenia, can be obtained from a search in the Medlars File. The drugs occasioning the publication of observations of thrombocytopenia during the period Jan. 1968 to April 1971 are listed in Table IX. These observations as well accord closely with the findings reported here, again with one exception. At the top of the list comes the combination of sulfonamide and folic acid antagonist which has been registered in several countries and is being tested in others for treatment of urinary tract infections (9 12, 17 18). The number of reports must naturally be judged in the light of the fact that it is a new compound, but may also suggest that special attention should be paid to the platelets when using this drug.

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CLINICAL VALUE OF KETONE BODY DETERMINATIONS IN BRITTLE DIABETES

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Abstract. The first part of this study is clinical investigation on the value of concomitant measurements of blood glucose and the ketone bodies for the improvement of control in 35 patients with brittle diabetes. Three groups are identified. 1) The keto resistant patient in whom only the blood glucose measurements are of value. 2) A group in whom about equally valuable information is obtained from blood glucose and the ketosis. 3) The Pedersen-Somogyi reactors in whom both blood glucose and ketonemia should be analysed. The level of ketosis may be judged from blood 3-hydroxybutyrate (3-HB) and acetoacetate (AcAc) or from breath acetone, although the latter does not change as fast as the metabolized 3-HB and AcAc. The changes in the urinary concentrations of 3-HB and AcAc are almost as rapid as those in the blood, and after insulin decrease in these urinary concentrations is often seen before the blood glucose begins to decrease. The second part of the study shows the positive correlations between the increase in blood glucose above 200 mg/100 ml of blood and blood 3-HB and AcAc and the decrease in the ratio blood 3-HB/AcAc with decreasing blood glucose. Preliminary data are also given on the decrement of blood 3-HB and AcAc with time, indicating an initial slower phase and a second faster one. The daily blood lactate changes were greater than expected. Initially they were parallel to the blood 3-HB and AcAc variations. Once blood glucose concentration also fell the lactate increased, mean of 1.9 mEq/l.

The name of the disease, diabetes mellitus, Zuckerkrankheit, as well as the laboratory facilities for measuring glucose have focused the interest on glucose at the expense of other parameters also affected in diabetics. As a rule, due attention to the blood glucose concentration is enough to obtain adequate control of the patient, but about 7 000 diabetics in Sweden (4) are in poor control as judged by a urine elimination of more than 50 g of glucose per day. The blood glucose level may be increased by several hormones but only decreased by one, i.e. insulin. An insulin effect on the blood glucose concentration may therefore be masked by one or several of the other factors that increase the blood glucose and, consequently, the net insulin effect may be difficult to discern, although a more detailed knowledge of this would be needed for determination of the optimal insulin dosage. The ketone bodies are formed when a minimum of glucose is not available to the tissues. As insulin promotes the entrance of glucose into the cells, it follows that an insulin action as measured from changes in the ketone bodies differs from an insulin action judged from changes in glucose, but may be of equal or even greater clinical significance.

The value of measuring breath acetone concentrations in diabetes has been demonstrated in previous reports (9, 10, 12, 20, 21). The method is rapid and reproducible and needs only a few ml of expired air. As acetone is dissolved in all the body tissues and not metabolized, it is slowly eliminated with a half-time of about 110 min (12). The changes in the acetone concentration will therefore probably not reveal changes in the degree of ketosis as rapidly as will the metabolized substances acetoacetate (AcAc) and 3-hydroxybutyrate (3-HB). The present study was therefore undertaken in order to try to establish the additional value of measuring AcAc and/or 3-HB in blood and/or urine in patients with diabetes, particularly those with brittle diabetes, as this is a clinical group which is difficult to manage and for which additional information over and above that on blood glucose may be of particular importance. In addition some diabetics not earlier on insulin were studied, as the effect of the insulin administration per se is best revealed after a single dose of insulin.

Table I *Clinical data on the 35 cases with brittle diabetes*

Abbreviations for insulin NL = Novo Lente, SL = Novo Semilente, NPH = Vitrum NPH, ZPI = Vitrum Zinc-protamine, RAP = Novo Rapitard, Crys. = Vitrum crystalline

Case no.	Age (yr)	Sex	Duration of diabetes (yr)	Insulin dosage on which the patients were in poor control (IU): Morning	Evening	Adequacy of insulin dosage: Too much	Too little
1	51	♂	6	32 NL			
2	78	♂	13	32 NL			
3	15	♀	7	24 RAP at 12.00 10 NL	16 RAP		
4	43	♀	16	40 NPH at 11.00 28 Crys.			
5	70	♀	14	20 NL			
6	37	♂	13	58 ZPI			
7	52	♂	13	36 NL			
8	48	♂	20	52 NL	28 NL		
9	17	♀	11	44 RAP	28 RAP		
10	43	♂	31	28 SL + 8 Crys.			
11	58	♀	29	48 NL	12 NL		
12	76	♀	17	44 NL + 20 Crys.			
13	55	♂	1/12	40 NL + 16 SL	16 NL		
14	23	♀	11	44 NPH		x	
15	54	♂	32	16 NL + 36 SL			
16	20	♀	1½	48 NL + 20 SL	32 NL		x
17	27	♀	11	28 ZPI	24 ZPI		
18	57	♂	39	34 NL + 12 Crys.			
19	38	♀	6	36 NL			
20	29	♀	23	40 NL			
21	43	♀	4	36 NL + 8 Crys.			
22	23	♂	6	24 NL + 20 SL			
23	29	♀	13	32 NL			
24	16	♂	2	36 NL			
25	23	♀	11	32 RAP	12 NPH		
26	54	♀	26	44 RAP			
27	41	♀	10	40 SL	24 SL		
28	22	♀	11	32 NPH	8 NPH		
29	[illegible]	♂	25	16 NL	36 NL		
30	34		20	26 NL Dibein 1 2			
31	45	♂	29	32 ZPI 8 Crys.			
	19	♂	12	28 NPH	16 NPH		
	19		1½	40 NL	24 NL		
	36		5	48 RAP			
	38		28	12 SL + 12 Crys.			
Mea	37.4		15.1				

MATERIAL AND METHODS

Forty-eight patients were studied. The majority, 35 patients, were classified as inadequately controlled. Most of them were referred from other hospitals because they were brittle, having had repeated episodes of hypo- and/or hyperglycaemia. Their usual, long-acting insulin was maintained. Another 13 diabetics were given single doses of fast-acting insulin or Novo Lente.

Data from the patients are summarized in Tables I and II. On 26 patients blood glucose and breath acetone measurements were done. In 22 cases serial measurements of 3-HB and/or AcAc were made on blood and/or urine during 3 to 24 hours. Venous blood for the 3-HB studies was obtained from an indwelling plastic catheter and the sampling presented no problem for the hospitalized patients. After instruction and training in the laboratory during the daytime two of the patients were able to take their night blood samples themselves at home.

The patients were on the standard hospital diabetic diet or on their usual home diet if studied ambulatorily. Of the 1 700 calories in the hospital diet 30% are from fat, 25% from protein and 45% from carbohydrates. The more active patients obtained a supplement of about equal calories of protein and carbohydrates. Although a similar diet was advised for the non hospitalized patients, inadequate information was obtainable about their food intake.

Blood glucose was analysed by the glucose oxidase method of Marks (10), 3-HB and AcAc with 3-HB dehydrogenase according to Williamson et al. (22), lactate enzymatically according to the Boehringer method. NH and total acid production by the kidneys were analysed chromatographically as described by Kihlberg (6) ... the

Table II. *Clinical data on 13 patients, earlier not on insulin, given single doses of insulin for study*

Abbreviations for insulin, see Table I

Case no	Age (y.)	Sex	Insulin (IU)
36	32	♀	20 NL
37	69	♀	20 NL
38	70	♂	20 NL
39	29	♀	10 Crys.
40	23	♀	20 Crys.
41	15	♀	20 Crys.
42	18	♂	28 NL
43	75	♀	24 Crys.
44	15	♀	20 Crys.
45	29	♀	20 Crys.
46	40	♂	20 Crys.
47	41	♀	20 Crys.
48	24	♀	12 Crys.
Mean	36.3		

Radiometer Titrator TTT 1 and Autoburette ABU 12. The blood glucose studies were made on capillary blood when acetone was measured and on venous blood in the 3-HB part of the investigation.

RESULTS

Clinical results

By an analysis of the blood glucose changes or by the additional study of the ketosis, 18 of the brittle patients were classified as having received too much and 16 too little insulin. Alternatively it was judged that the dosage of insulin was adequate but that the time of administration should be changed or the dose divided into two.

In discussing the relative merits of glucose versus ketosis studies in the control of diabetes the patients may be divided into three groups. In the first would fall patients as exemplified in Fig. 1 for whom no important information was obtained from the 24-hour studies of 3-HB or AcAc, either in the blood or in the urine. On that day the urine contained only traces of keto acids. Disregarding for the moment the interesting information that the patient was keto resistant, the measurements of 3-HB and AcAc were not of any value in directing the changes in the insulin therapy. The blood glucose curve alone in Fig. 1 tells that the morning insulin dose should be reduced in order to prevent the hypoglycaemia in the afternoon.

To a second group would belong such a case as illustrated in Fig. 2. Here basically the same information was obtained from the blood glucose curve as from the 3-HB or AcAc curves, including that of the urine. All these curves suggested an insufficient insulin action in the morning. The blood glucose curve in the afternoon might suggest an insufficient insulin action, but this is not revealed in any increase in 3-HB or AcAc. Instead of the 40 IU Novo Lente insulin in the morning, she was given 32 IU Novo Lente in the morning and *16 IU* in the evening and was subsequently in better control and could be discharged.

Finally in a third group of cases adequate information was only obtained from the analysis of both blood glucose and the ketosis. To this group belonged those patients who getting too much insulin had hypoglycaemia and subsequently ketonaemia as seen in Fig. 3 *a*. This patient had hypoglycaemia at noon and in the afternoon his

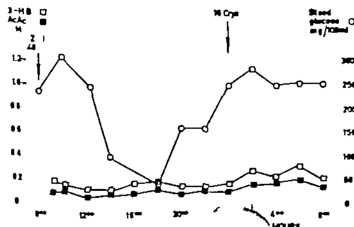


Fig. 1. 24-hour curves of blood glucose, *3-HB and AcAc in the keto resistant patient no. 35.*

breath acetone and blood glucose concentrations increased. This *hypo-hyperglycaemic swing* could not be identified with certainty in some of the cases as long as the study was only undertaken during office hours.

If both the keto acid and the blood glucose were high in the morning and gradually became normalized during the course of the day the patient could either have too little insulin during the night or too much with a subclinical hypoglycaemia in the early hours of the morning. The true state of affairs may be revealed either by trial and error in changes of the insulin dosage or by a 24-hour study The latter could then be limited to blood glucose determinations. Alternatively the case may be interpreted from the *urine glucose content in the manner that, if the* patient has had too little insulin the morning urine would contain relatively *much glucose* whereas if the patient has had too much insulin during the night, his morning urine would contain little or no glucose in spite of the high morning blood glucose level.

It was observed in two of the patients who had had hyper hypoglycaemic swings with ketonaemia that, when their insulin dosage was changed and they improved, their post-hypoglycaemic ketonaemia did not occur any more in other words they had become relatively keto resistant (Fig. 3 *b*)

In contrast to the rest of the group the con*trol of two patients was difficult to achieve*. It took some 10 weeks before the hypoglycaemic *swings of patient no 30 disappeared. This could* ly be accounted for by an instability due to fection partly to her mental lability which sometimes led her to eat very little without informing the staff The other patient, no 35 was hospitalized for a total of 7 months out of 9 after his first admission to our hospital. The initial reason was severe attacks of hypoglycaemia, but in addition his blood sugar varied from 100 to 600 mg/100 ml during the day or from day to day without any apparent reason and was seemingly not much influenced by the insulin dosage. 24-

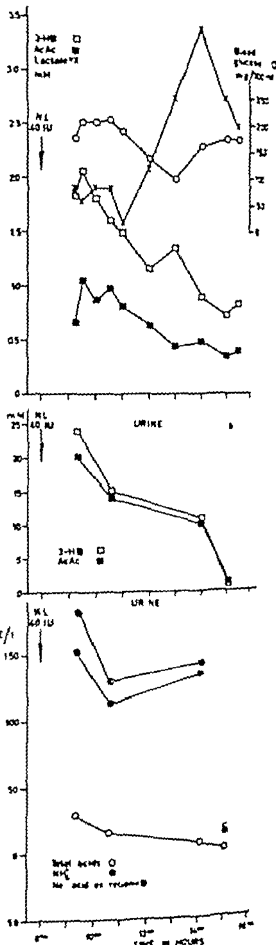


Fig 2 Patient no. 33. (*a*) Blood glucose, lactate, 3-HB and AcAc curves. (*b*) Urinary concentrations of 3-HB and AcAc at the same time. (*c*) Urinary concentrations of total acids, NH and net acid excretion during the same time.

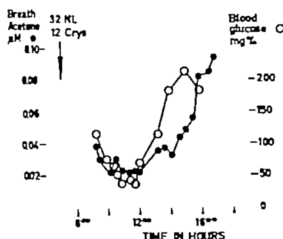


Fig. 3 a. Blood glucose and breath acetone in patient no. 31 with hypo-hyperglycaemic "swing". Note his increase in acetone after the hypoglycaemia.

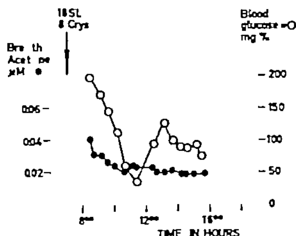


Fig. 3 b. Same patient as in 3 a. After reduction of his morning insulin dosage he had no ketosis in spite of the hypoglycaemia.

hour studies were made on two occasions. The first time happened to be one of the few days during his hospital stay when his blood glucose was not elevated (maximum 109 mg/100 ml). At noon he showed clinical signs of hypoglycaemia and had a value of 42 mg/100 ml, but there was no increase in his ketonaemia. The second occasion is shown in Fig. 1. As will be seen, he had a high morning blood glucose level, was hypoglycaemic at 18.00 but showed no clinical signs of this, and later his hyperglycaemia returned. In spite of the hypo- and hyperglycaemia the blood concentrations of 3-HB and AcAc remained virtually constant and within the normal levels. This patient then differed from the others in being both keto resistant and having labile blood glucose levels.

The clinical experience from this part of the study has been that the insulin dosage could in principle be as well decided on the basis of measurements of blood 3-HB or AcAc as of blood glucose. The close relation between the AcAc and the 3-HB changes indicates that it is enough to measure one of these acid residues, preferably 3-HB as being the most stable substance and as showing the greatest changes.

Serial blood and urine measurements of 3-HB or AcAc were performed on 10 of the patients. On the whole the results were consistent and in accordance with Fig. 2. The urine concentrations of the keto acids varied in parallel with the blood changes, if these were elevated. Although the changes in the blood concentrations appeared earlier than those in the urine, the ketosis may for all clinical purposes, be evaluated from urine measurements of 3-HB. A more detailed report of the kidney elimination of AcAc and 3-HB is given elsewhere (14). A separate study on the relation between blood 3-HB and AcAc and plasma glycerol and FFA in seven of these patients will be reported (15).

If the ketosis was sufficiently pronounced, the level of ketonaemia could also be evaluated from measurements of the titratable acids, as also seen in Fig. 2. Although the level was not established with precision, it seems that when the 3-HB + AcAc concentration in the urine were < 10 mEq/l no significant changes in the titratable acids in the urine were found.

Experimental results

Relation between blood glucose level and ketosis

Fig. 4 demonstrates the close correlation between the blood glucose level and the concentrations of both 3-HB and AcAc in the blood provided that the blood glucose is above a value of about 200 mg/100 ml. This correlation is valid both in a direct plot, where the values were between 0.60 and 0.96 and in a semilog plot. It will be seen that the slope of the 3-HB and AcAc curves were similar but that the intercept differed. The range observed may therefore be attributed both to individual variations from day to day and to interindividual differences.

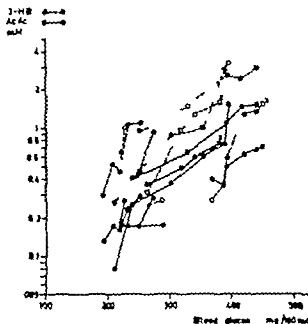


Fig 4 Relation between the blood glucose concentration and the log blood 3-HB and AcAc concentrations in patients whose ketosis was decreasing. denotes a single patient, no. 23 studied on two different days.

The data in Fig 4 all refer to a decreasing ketonaemia and decreasing glycaemia. As the *rates of change of the blood glucose and of the* 3-HB and AcAc concentrations differ dissimilar curves will be obtained during increasing and decreasing ketosis When the ketosis increases, the slope of the curves tends to be more horizontal.

Fig. 5 exemplifies the decrease in the blood 3-HB and AcAc concentrations and the decrease in the 3-HB AcAc ratio after a single dose of insulin.

Disappearance rate The available data may be used to give an idea of the rate of the decrease of 3-HB and AcA in the blood after the onset of insulin action in diabetics Fig 6 shows the log of the decrement plotted against time Increment is the actual value minus the normal level and is mathematically better suited to study kinetics than the actual value itself Decrement is the same entity as increment but a preferable term when working with decreasing concentrations. A straight line drawn for the glucose values gives a $T^1/_2$ of 90 min corresponding to a k value of 1.4 The disappearance rate of both 3-HB and AcAc is faster The same patient studied on another day exhibited a slower decrease of her blood glucose—$T^1/_2$ 210 min—but the $T^1/_2$ 3-HB and AcAc were of the same order of magnitude as shown in Fig. 5 The initially higher $T^1/_2$ values followed by a more rapid decrease seemed to be the rule in the cases studied, the rapid phase having a $T^1/_2$ of about 15 min.

Blood lactate The blood lactate concentration varied considerably during the day rising from about 1 mEq/l to 3 or 4 mEq/l and in one case to 6 mEq/l although all the patients were at rest at the time of sampling. Two phases of a pattern could be recognized. Although not too distinctly in two of the cases, the blood lactate concentration fell at about the same time as plasma 3-HB and AcAc began to decrease after the insulin administration, as exemplified in Fig. 2. During this time the blood glucose remained stable but once the glucose level fell, the lactate concentration increased in all cases and there was a statistically significant correlation between these parameters ($r = 0.8$, $n = 10$ $p < 0.01$). The lactate concentration thus increased irrespectively of the level of ketonaemia and was of the same order of magnitude in the keto resistant patient and in those who only had a small increase in 3-HB If the time from the lowest to the highest lactate concentration was studied, it was seen that the mean increase in lactate during this period was 1.9 mEq/l and the mean decrease in the sum of 3-HB and AcAc was also 1.9 mEq/l.

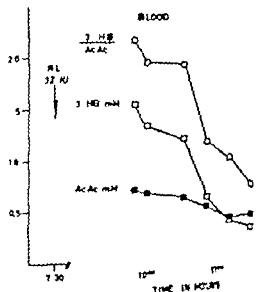


Fig. 5 Changes in the ratio 3-HB/AcAc and the blood concentrations of 3-HB and AcAc in patient no. 23

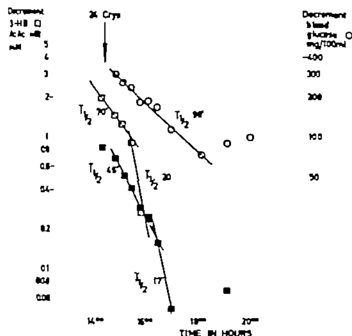


Fig. 6. Disappearance rate of blood glucose 3-HB and AcAc in patient no. 3

DISCUSSION

The present study of ketosis in diabetes has not been directed to instances of manifest keto acidosis in the traditional clinical sense but to the value of ketone measurements in the control of the patients and to the diurnal variations in the level of ketosis in diabetics. Earlier studies (17 22), also covering in some instances 24 hours of the day showed that the level of ketonaemia may vary considerably as in the present study

It is important to recognize and to reduce a pathological ketonaemia, because if only the blood glucose is elevated the metabolic disturbance is less serious than when both the blood glucose and 3-HB and AcAc concentrations are increased (23).

As a guide to optimal control of the diabetic patient the integrated analysis of blood glucose and blood 3-HB and/or AcAc is preferable to that of blood glucose alone (7 22, 26, 27) The ketosis may be monitored in several ways, as the present study has shown. 24-hour studies of blood glucose and 3-HB give the best data for establishing good control. Similar information will be obtained in urine 3-HB analysis Finally 3-HB measurements may be substituted by those of breath acetone in many cases.

As judged from the present experience, such multiple analysis would improve the state of control of most diabetic patients, and very few should be left in poor control However it is difficult to ascertain for how many of the diabetic patients such a detailed study of the ketone bodies is indicated. At the beginning of the present investigation the 3-HB and AcAc data were probably more often decisive for a change in the insulin administration than later when, as a result of the intensive study we had learned to interpret the blood glucose data better and to make 24-hour observations more frequently

In the literature the hypo-hyperglycaemic swing associated with ketosis is often called the Somogyi (18) effect, although it was first described by Poulsen (11). Recently the clinical importance of this swing was stressed (1 22). That the tendency to a Poulsen-Somogyi reaction may be reduced when the patients come under better control was demonstrated by two patients in the present series

Quantitative correlations between the concentrations of blood glucose and ketosis are scarce in the literature and could not be expected prior to the introduction of the enzymatic method of ketone bodies. Werk & Knowles (23) demonstrated *positive correlation between the level of* blood glucose and ketosis in their tables, but a negative correlation in repeated measurements on 17 patients. Tassopoulos et al. (21) found a posi-

tive correlation between the blood glucose and breath acetone concentrations. The present results show that it is necessary to study the individual case and to take into account whether the ketosis is increasing or decreasing. When this was done a strong positive correlation was found between blood AcAc and 3-HB on the one hand and blood glucose on the other

For an unbiased analysis of the disappearance rate of a blood or plasma constituent the data should be treated in the same way as Hlad and Elrick (5) dealt with blood glucose Unfortunately in the present material it was found that the number of observations for 3-HB and AcAc was not sufficient for such a calculation as the keto acids did not continue to decrease long enough. The disappearance rate was therefore only expressed as decrement half time. The slower initial phase and the faster second phase were consistently observed but the discussion of the causes for such a difference in $T^1/_2$ times must be speculative One explanation could be that after insulin administration the utilization is initially increased and that subsequently the liver production of 3-HB and AcAc decreases only when the stored surplus of FFA in the liver is metabolized From the rapid drop in plasma FFA and glycerol after insulin injection (15) it would otherwise have been expected that 3-HB and AcAc should decrease equally fast, glycerol having a $T^1/_2$ of 10 to 14 min (2, 16)

Galvin et al (3) found a constant ratio of plasma 3-HB AcAc during the decrease of ketosis caused by starvation and post exercise By contrast, Rooth et al (16) showed that when the ketosis of starving obese adults was reduced after glucose administration the ratio 3-HB AcAc fell, and identical results were obtained (8) when the ketonaemia of fasting was reduced by the ingestion of nicotinic acid or by salicylic acid The present result show that the ratio also fell when the ketonaemia of the diabetic was reduced by insulin. A reduction in the ratio 3-HB AcAc probably reflects an increased β-oxidation of fatty acids in the liver and an increase in the ratio NADH/NAD in the liver mitochondria (8)

Although in the present study the decrease of ketonaemia was studied more than the increase the data obtained suggest an increase in the ratio 3-HB/AcAc when the level of ketonaemia augments. This, again, would be in accordance with our earlier studies of ketonaemia in fasting obese and normal subjects (14).

During ketosis the entry of pyruvate into the Krebs cycle is partly blocked (24), and pyruvate and lactate intra and extracellular concentrations increase After the onset of the insulin effect the blood lactate concentration begins to decrease at the same time as that of 3-HB and AcAc. However soon afterwards the lactate concentration again increases, now at the same time as the glucose concentration decreases. This could be explained as the result of an increased availability of glucose in the cell. The increase in blood lactate sometimes observed after phenformin could perhaps also be explained on a similar basis. The magnitude of the lactate changes is considerable in spite of the modest ketonaemia in the cases studied and a knowledge of these levels is useful to prevent confusion with true lactacidosis the term then used in the clinical sense and implying a severe diseased state of a patient. It is interesting to see that even the keto resistant patient, and those with little ketonaemia during the study had an increase in lactate at the time of the fall of the blood glucose concentration. This increase consequently was not dependent upon the level of ketosis.

Keto resistance is obviously a relative concept. As stated, two patients were keto prone at the time when they had a marked Poulsen-Somogyi reaction but later became relatively keto resistant when they were in better control. One case appeared to be entirely keto resistant at the time of the 24-hour studies, but twice during the last year he was admitted to the hospital in diabetic coma with pronounced keto acidosis. Also the tendency to ketosis is reduced with age There seems to be a gradual transition from the keto prone starving infant to the young diabetic and further to the keto resistant maturity onset diabetic, and finally on to the hyperosmolar non-ketotic diabetic coma patient, but a precise knowledge of what factor makes the patients more keto resistant is still lacking

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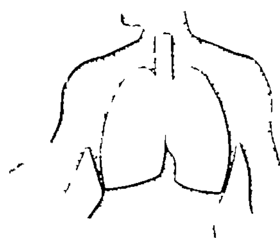
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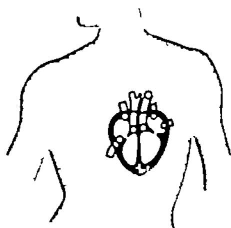
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Acta med. scand. Vol. 191 pp. 599–563 1972

DIURNAL VARIATIONS IN BLOOD GLUCOSE, 3-HYDROXYBUTYRATE, ACETOACETATE, PLASMA FREE FATTY ACIDS AND GLYCEROL IN DIABETICS

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Abstract. 4-hour studies of the changes of blood glucose, 3-HB, AcAc, plasma FFA and glycerol have been made five diabetics on long-acting insulin and 10-hour studies in two patients receiving short-acting insulin. The reduction in the concentration of 3-HB, FFA and glycerol d that of glucose. AcAc initially increased after the administration of insulin. The reappearance of ketonaemia is preceded by an increase in plasma FFA and glycerol by 3 to 4 hours. One patient who was keto-resistant showed no significant increase in plasma FFA or glycerol concentration either during hyper- or hypoglycaemia.

In 1956 Dole (2) and Laurell (6) independently described an increase in the plasma free fatty acid (FFA) concentration in patients with diabetic ketosis. It has been established that 3-hydroxybutyrate (3-HB) and acetoacetate (AcAc) are produced in the liver from FFA mobilized from adipose tissue. During this mobilization glycerol is also released and, as it is not metabolized by the peripheral tissues like FFA, its changes of concentration reveal the lipid mobilization better than FFA (4, 14). In spite of the intimate relationship between FFA and glycerol, on the one hand, and 3-HB and AcAc, on the other, few studies have been made in diabetics on the pattern of changes of these parameters.

MATERIAL AND METHODS

The patients were all treated at the Medical Department A, University Hospital, Lund. Two diabetics who received insulin for the first time were observed for 10 hours and five brittle diabetics were studied during 24 hours. Serial measurements were made of blood glucose, 3-HB, AcAc, plasma FFA and glycerol.

Blood glucose was analysed by the glucose oxidase method of Marks (9), 3-HB and AcAc by the 3-HB dehydrogenase method of Williamson et al. (16), plasma

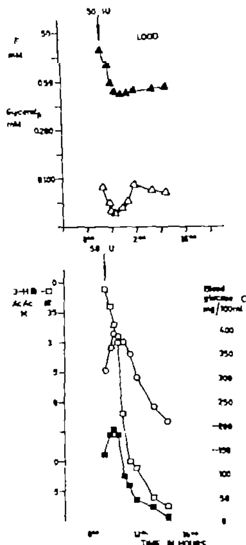


Fig. 1. Plasma FFA and glycerol, blood glucose, 3-HB and AcAc curves from diabetic patient who previously was not on insulin. 50 IU of rapid acting sulphated insulin were given at arrow.

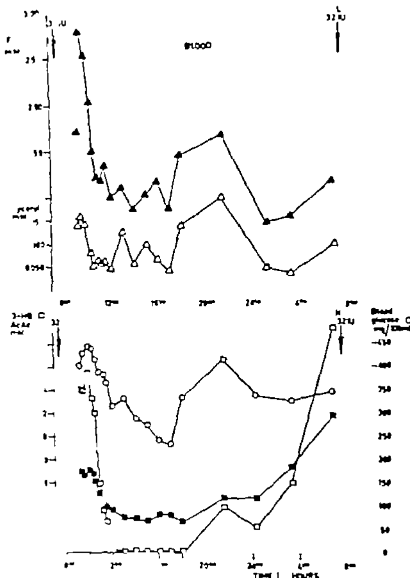


Fig 2. 24-hour curves in a diabetic patient receiving 32 IU Lente insulin. Note that the drop in the blood glucose concentration appears late but that the afternoon increase in the blood glucose level precedes that of plasma FFA and glycerol.

[illegible] method of Laurell and Tibbling (8), and [illegible] of Laurell and Tibbling (7). All measurements [illegible] in blood drawn from an indwelling [illegible] cubital vein. The patients were taking the [illegible] diabetic diet and none was in bed except

RESULTS

Figs 1, 2 and 3 illustrate the changes in the parameters when the level of ketonaemia was initially elevated, blood 3-HB being above 1.0 mM. The normal level is about 0.2 to 0.4 mM. The decrease in all the measurements is best seen after the effect of the short-acting insulin, as shown in Fig. 1. Within 30 min blood 3-HB, plasma FFA and glycerol decreased, whereas the blood glucose concentration began to decrease later. Initially the blood AcAc concentration might rise but then fell more or less at the same rate as 3-HB. In the patients receiving the long-acting insulin the pattern of changes was similar but the fall began two to three hours after the insulin administration.

In two of the patients studied the blood glucose level remained below 200 mg/100 ml of blood and there was no increase in the level of ketonaemia; neither was FFA nor glycerol elevated, nor could any systematic changes be seen.

Finally in the case shown in Fig. 4 the blood

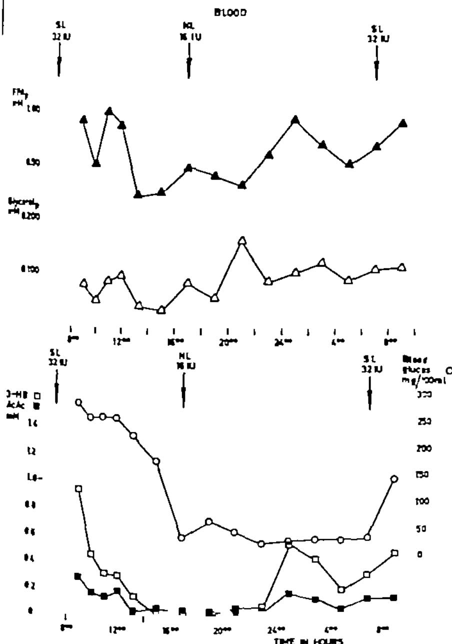


Fig. 3. Same parameters as in Figs. 1 and 2. Note the late appearance of the ketonaemia after the hypoglycaemia. 3 IU Semilente insulin were given in the morning and 16 IU Lente insulin in the afternoon.

glucose varied between 300 and 50 mg/100 ml of blood during the day but in spite of this neither blood 3-HB, AcAc nor plasma FFA varied much or exceeded the normal levels.

The study was initially focused on the decrease of the ketonaemia, but in three of the cases studied for 24 hours there was an increase in the ketonaemia in the afternoon or during the night, so that the time sequence of the reversal of the changes could be studied. When the blood glucose concentration again began to increase as in Fig. 2, plasma FFA and glycerol followed some 30 min later but the increase in blood 3-HB and AcAc only appeared 3 to 4 hours after the blood glucose increase. The same delay was seen when, as in Fig. 3 the ketonaemia was initiated by hypoglycaemia. The peak of the plasma glycerol concentration came at 21 hours and the peak of the blood 3-HB level 4 hours later. The same interval was also noted in the third patient.

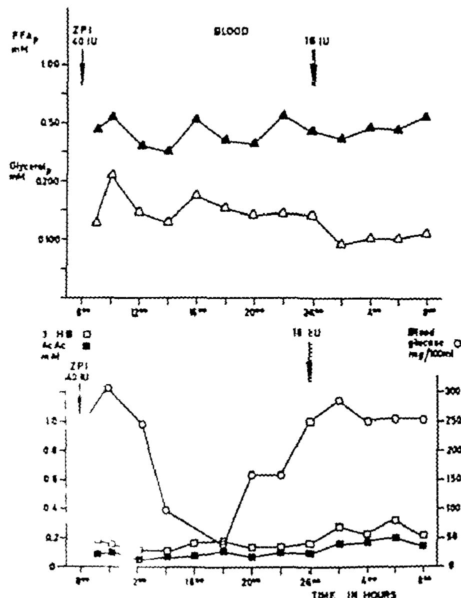


Fig. 4. The keto-... tient. 40 IU Zn-prota... lendes ... are given in ... morning and 16 IU cry... line insulin at 4 ... Note the small ... 3-HB and AcAc, and F...

DISCUSSION

The fact that ... the blood glucose concentration but ... the degree of ketonaemia, may vary during the day in diabetics was observed by Poulsen (11) ... early a 1941 and has later been confirmed by W...kin ... et al. (15). Besides this observation the present ...esults show the time relationship between 3-HB and AcAc and their precursor FFA. Earlier investigations on the time sequence of changes between FFA and glycerol on the one hand and 3-HB and AcAc, on the other have dealt with experimental FFA decrease after nicotinic acid administration or glucose injection (1, 13). The changes seen by these authors were similar to those now observed after insulin administration. The rapid decrease in FFA ... glycerol concentrations probably reveals the ... hibition of the lipid mobilization caused by ... sulin. The subsequent or simultaneous drop ... 3-HB concentration indicates the reduced pro... tion of 3-HB in the liver. Just as when the ... mobilization was reduced in starving, ketotic ... jects by glucose or nicotinic acid or acetylsa... acid administration the AcAc concentration ... tially increased before it dropped (5, 13).

The time sequence between the FFA and ... cerol increase and the AcAc and 3-HB augmen... tion in the diabetic patient does not seem to ... been reported earlier except by Dole (3) ... states that the FFA increase precedes that of ...

ketonaemia. Kågedal et al. (5) observed the effect of nicotinic acid on starving normal and obese subjects. The rebound increase in FFA and glycerol anticipated that of 3-HB and AcAc by at least two hours. This time interval is of the same order of magnitude as McPherson et al. (10) found between the onset of hypoglycaemia and the peripheral vein concentration of total blood ketones. As there is an interval 3 to 4 hours between the lipid mobilization, as revealed by the FFA and glycerol increase and the appearance of the ketonaemia, it follows that, for instance, a morning ketonaemia may exist with or without much increase in FFA and glycerol.

The keto-resistance in the patient illustrated in Fig. 4 is explained by the observation that the degree neither of hyper nor hypoglycaemia, that he had during the day of observation, was sufficient to induce an increased lipid mobilization. Consequently no substrate for an increased 3-HB or AcAc production was offered.

ACKNOWLEDGEMENTS

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TUBULAR REABSORPTION OF PHOSPHATE AND CALCIUM IN PRIMARY HYPERPARATHYROIDISM

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Abstract. Phosphate clearance (Cl_{Ph}) and the renal tubular reabsorption of phosphate and calcium (TRP and TRCa) have been determined in 90 patients, 31 with primary hyperparathyroidism (PHP), 9 with non-parathyroid hypercalcemia, 24 with nephrolithiasis and 26 with other diseases in order to determine the value of these parameters in diagnosing PHP. A high Cl_{Ph} (>15 ml/min) was seen more commonly in patients with PHP than among patients in the other groups. Measurement of this parameter, however, was not found to be of any definite value in diagnosing PHP. Renal TRP < 80% was found in 35.5% of patients with PHP, in 33.3% of patients with non-parathyroid hypercalcemia, in 0% of patients with nephrolithiasis and in 11.5% of patients with other diseases. The TRP, however, was found to vary with creatinine clearance (Cl_{cr}) and thus the TRP could be used as diagnostic parameter of PHP in patients with normal Cl_{cr} (>70 ml/min). The renal TRCa was found to be of aid in diagnosing PHP in patients with greatly increased serum calcium values. Thus complete differentiation was found between patients with PHP and patients with non-parathyroid hypercalcemia when the TRCa was compared with the serum calcium concentration.

In making a diagnosis of primary hyperparathyroidism (PHP) two situations may be met. 1) A normal to questionable increased serum calcium concentration may be present, thereby presenting a *differential diagnostic problem* as regards a series of normocalcemic, normoparathyroid conditions with symptoms similar to PHP (nephrolithiasis, gastric and duodenal ulcer, non-characteristic dyspepsia, pancreatitis and certain nervous conditions). 2) A clearly increased serum calcium concentration may be present, making it necessary to rule out a series of hypercalcemic hypoparathyroid conditions (sarcoidosis, multiple myeloma, bone metastases, immobilization, milk alkali syndrome, etc.).

In the present study renal phosphate clearance (Cl_{Ph}) together with the tubular reabsorption of phosphate (TRP) and calcium (TRCa) were determined in 90 patients in an attempt to evaluate the value of these parameters in the diagnosis of PHP. In order to elucidate the above mentioned differential diagnostic problems, patients with PHP, nephrolithiasis without evidence of hyperparathyroidism and patients with non-parathyroid hypercalcemia were included. In addition, a heterogenic group of hospitalized patients was examined.

MATERIAL

Ninety patients, admitted to the medical or surgical departments of Arhus County Hospital during the period 1959–71 were studied. The patients were separated into 4 groups: 1) patients with PHP, 2) patients with non-parathyroid hypercalcemia, 3) normocalcemic patients with nephrolithiasis and 4) patients with other diseases. The age and sex of the patients studied are given in Table I.

Group 1. Primary hyperparathyroidism. Thirty-one patients with PHP were studied. In 29 of these patients the diagnosis was confirmed by operation and in one parathyroid adenoma which was thought to be malignant was found at autopsy. In 27 of the operated patients parathyroid adenoma was found and in 2 patients hyperplasia of all four parathyroid glands. In the last patient macroscopically as well as microscopically normal parathyroid glands were found on exploration of the neck. This patient had constant hypercalcemia, also after operation, together with low to normal serum phosphate. In addition, the patient had both epulis and bone cyst, which on microscopic examination revealed osteitis fibrosa, and this patient has therefore been included in the study group. Furthermore this patient became normocalcemic after an additional neck exploration.

Group 2. Non-parathyroid hypercalcemia. Nine patients were studied, three of whom had thyrotoxicosis. Serum

Table I *Age and sex distribution of the patients studied*

	Females	Males	Age (y)
PHP	18	13	21-75
Non-parathyroid hypercalcemia	4	5	39-67
Nephrolithiasis	7	17	21-58
Other diseases	14	12	11-77

calcium became normal after strumectomy. Two patients had Boeck's sarcoidosis with steroid-suppressable hypercalcemia. One patient with multiple fractures developed hypercalcemia during immobilization. In addition there was one patient with vitamin D poisoning, one with mammary cancer and bone secondaries, and one with pheochromocytoma in whom the serum calcium concentration became normal after its removal.

Group 3. Nephrolithiasis. Twenty-four normocalcemic patients with nephrolithiasis were studied.

Group 4. Other diseases. In this group 26 patients with the following diagnoses were examined: thyrotoxicosis (1 pat.), Boeck's sarcoidosis (? pats.), malabsorption (5 pats.), observation without therapeutic indication (? pats.), myoses variae (1 pat.), lipothymia (1 pat.), ambustio faciei et crurs (1 pat.), anxiety and neurasthenic neurosis (? pats.), gastritis (? pats.), medullary sponge kidney (1 pat.), bilateral hydronephrosis (1 pat.), essential arterial hypertension (1 pat.), chronic pyelonephritis (? pats.), non-toxic goitre (1 pat.), treated thyrotoxicosis (? pat.) and fractura corporis femoris san. sequelae (1 pat.). Hypercalcemia was not present in any of these patients.

METHODS

Studies were carried out over a period of 6 days during which the patient received a standard diet consisting of ?000 calories per day. This diet contained 135 mg calcium, 775 mg phosphate and 693 mg sodium. Urinary output during days 4, 5 and 6 were first measured, then mixed and the urinary concentrations of phosphate, calcium and creatinine were measured. On the morning of the 5th day the fasting serum concentrations of phosphate, calcium and creatinine were determined. With the use of these values phosphate clearance (Cl_{pi}), calcium clearance (Cl_{Ca}) and creatinine clearance (Cl_{cr}) were calculated. At the beginning of this study (until 1.1.69) calcium was determined by EDTA titration (1) and later by flame photometry (Eppendorf flame photometer). Phosphate was determined using the molybdenumblue method (10) and creatinine was determined according to the Jaffe reaction after absorption with Lloyd' reagent.

TRP was calculated according to the formula

$$TRP = \left(1 - \frac{Cl_{pi}}{Cl_{cr}}\right) \times 100\,\%,$$

and TRCa according to the formula

$$TRCa = \left(1 - \frac{Cl_{Ca}}{Cl_{cr}}\right) \times 100\,\%$$

RESULTS

Cl_{pi}. The values for Cl_{pi} from all of the patients studied are given in Fig. 1. As shown in the figure the Cl_{pi} in patients with PHP was higher than in the other three groups, but on the other hand there was a rather large range of values. As the values did not show a normal distribution it was decided to determine whether high phosphate clearance values were as common in patients with PHP as in patients in the other groups. It was arbitrarily chosen to determine whether Cl_{pi} $\geq$ 15 ml/min appeared as commonly in patients with PHP as it did in the other groups of patients. $Cl_{pi} \geq 15$ ml/min was found in 32 % of patients with PHP, in 22 % with non-parathyroid hypercalcemia, in 21 % with nephrolithiasis and in 1 % with other diseases. No statistically significant differences exist between these values (Fischer's exact test, $\alpha > 0.20$).

Cl_{pi} measured as 4-hour clearance could not be used to separate patients with PHP from patients in the other groups.

TRP in all of the 90 patients studied is given in Fig. 2. As the values were not normally distributed, it was arbitrarily decided to determine whether TRP less than 80 % was as common

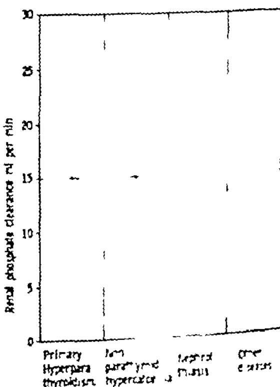


Fig. 1. Renal Cl_{pi} in all 90 patients studied.

in patients with PHP as in the other patients. The borderline value of 80% is often given in the literature. TRP < 80% was found in 11 (35.5%) of the patients with PHP as contrasted with 6 (10.2%) of all of the other 59 patients. This value was found to be statistically significant (0.01 > p > 0.001).

As shown in Table II, a TRP less than 80% was seen with approximately the same frequency in patients with PHP and in patients with non-parathyroid hypercalcemia (Fischer's exact test 2 α > 0.20). The reason for this may perhaps be related to the small number of patients with non-parathyroid hypercalcemia. In patients with nephrolithiasis a TRP < 80% did not appear and in patients with other diseases a TRP less than 80% was found in 11.5%. These values differed significantly from the value in patients with PHP (Fischer's exact test 2 α = 0.002 and 2 α = 0.05).

TRP and Cl_{cr}. The 6 patients without PHP in whom a low TRP was found, all had a low Cl_{cr}

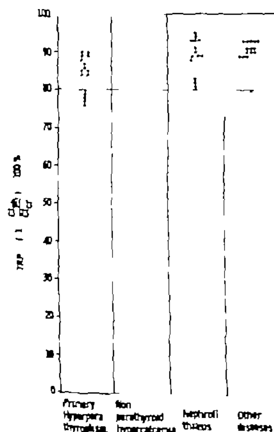


Fig. 2. TRP in all 90 patients studied.

Table II *TRP in all 90 patients studied*

	TRP < 80%		TRP > 80%	
	(n)	(%)	(n)	(%)
PHP	11	35.5	20	64.5
Non-parathyroid hypercalcemia	3	33.3	6	66.7
Nephrolithiasis	0	0	24	100
Other diseases	3	11.5	23	88.5

(6–56 ml/min). In 8 of 11 patients with PHP and TRP less than 80% Cl_{cr} was found to be less than 70 ml/min. A comparison between Cl_{cr} and TRP is given in Figs. 3 and 4.

As shown in Fig. 3 there was a relationship between Cl_{cr} and TRP in patients with PHP in that a low TRP was found in patients with a low Cl_{cr}. This relationship was statistically significant (Spearman's rank correlation, $R = 0.691$, $\alpha < 0.001$).

A similar situation was found in the 59 patients without PHP (Spearman's rank correlation, $R = 0.401$, $\alpha < 0.01$). Since TRP appeared to be dependent upon Cl_{cr}, the data were examined to determine whether a low TRP was commoner in patients with PHP and a normal Cl_{cr} than in other patients with a normal Cl_{cr}, the limit of normal for Cl_{cr} being set at 70 ml/min. In 47 patients without PHP with $Cl_{cr} \geq 70$ ml/min, TRP values less than 80% were not seen, whereas a TRP less than 80% was found in 3 of 20 patients with PHP and $Cl_{cr} \geq 70$ ml/min (15%). This difference was statistically significant ($p = 0.0238$).

Thus TRP appears to be dependent upon glomerular filtration as measured by Cl_{cr}. In patients with a normal Cl_{cr} (≥ 70 ml/min) the determination of TRP appears to be of help in diagnosing PHP.

TRCa. Values for TRCa are given in Fig. 5. As the values were not normally distributed, the differences between groups were studied, distribution about an arbitrarily chosen borderline value of 98% being studied. TRCa greater than 98% was found in 17 (55%) of patients with PHP as contrasted with 2 (22%) of patients with non-parathyroid hypercalcemia. This difference was not statistically significant (Fischer's exact test 2 α = 0.20). In 23 (96%) of patients with nephro-

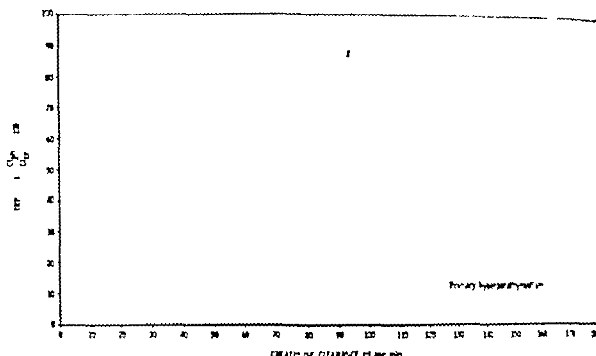


Fig. 3. Correlation between TRP and Cl_{cr} in 31 patients with primary hyperparathyroidism (Spearman's rank correlation, $R = 0.69$, $\alpha < 0.001$).

lithiasis and in 23 (88%) with other conditions the TRCa was found to be greater than 98%. These groups of patients differed significantly from patients with PHP (Fischer's exact test $2\alpha < 0.002$ and $2\alpha < 0.01$), TRCa in these patients being greater than in patients with PHP. There was, however, a good deal of overlap between the groups.

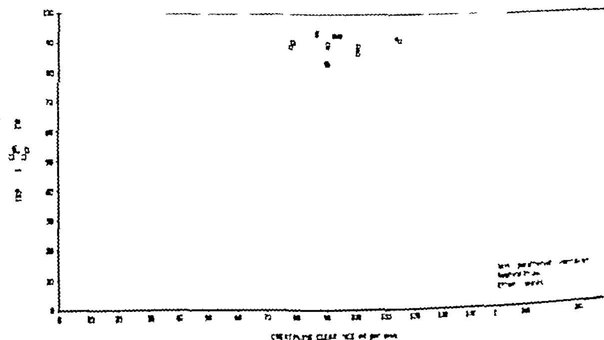


Fig. 4. Correlation between TRP and Cl_{cr} in 9 patients with non-parathyroid hypercalcemia, 4 with nephrolithiasis and 26 with other diseases (Spearman's rank correlation, $R = 0.40$, $2\alpha < 0.01$).

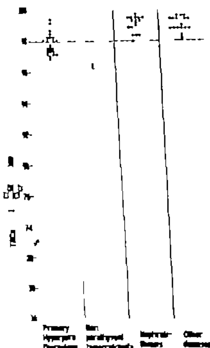


Fig. 5. TRCa in all 90 patients studied.

There was no significant difference between the nephrolithiasis group and the group of patients with other diseases (Fischer's exact test 2 α > 0.20).

TRCa and serum calcium. In patients with PHP a relationship was found between serum calcium and TRCa (Fig. 6) high serum calcium values being accompanied by a low TRCa. This correlation was statistically significant (Spearman's rank correlation, $R = -0.50$ 2 α < 0.01)

Patients with non-parathyroid hypercalcemia generally had lower TRCa values than hypercalcemic hyperparathyroid patients with the same serum calcium value. A line parallel with the line of regression for serum calcium greater than 2.75 mmol/l on the graph completely separated the two groups of TRCa values. The three patients with non-parathyroid hypercalcemia whose TRCa values were in the area above this line were the two patients with thyrotoxicosis and a patient with pheochromocytoma. They all had serum calcium values less than 2.75 mmol/l but greater than 2.70 mmol/l. The laboratory's normal range for serum calcium is 2.35–2.70 mM ($M \pm 2$ S.D.)

TRCa values for the nephrolithiasis patients did not differ from the values seen in patients with slight hypercalcemia, irrespective of whether

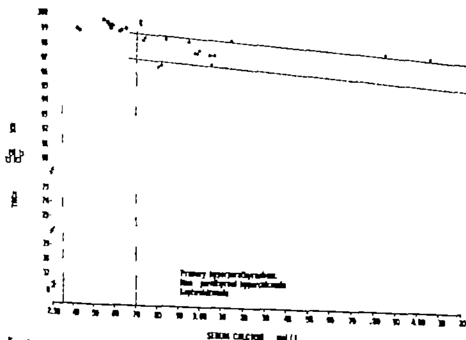


Fig. 6. Correlation between serum calcium concentration and TRCa in 31 patients with primary hyperparathyroidism, 9 with non-parathyroid hypercalcemia and 24 with nephrolithiasis. The solid line represents the line of regression (TRCa = −1.2 serum calcium concentration + 101.9). The stippled line was drawn arbitrarily parallel to the line of regression. The area of normal calcium concentration lies between the vertical stippled lines.

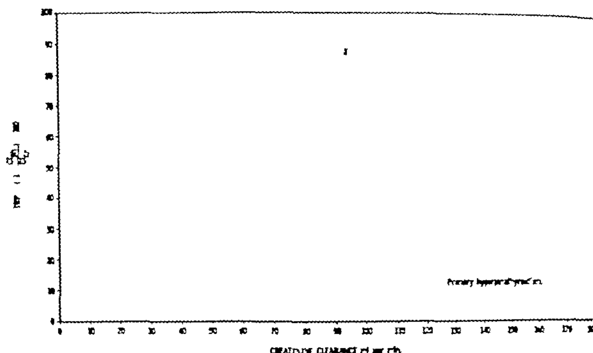


Fig. 3. Correlation between TRP and Cl_{cr} in 31 patients with primary hyperparathyroidism (Spearman's rank correlation, $R = 0.69$, $\alpha < 0.001$).

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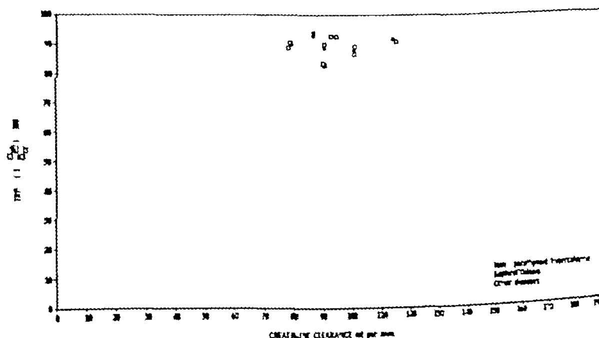


Fig. 4. Correlation between TRP and Cl_{cr} in 9 patients with non-parathyroid hypercalcemia, 4 with nephrolithiasis and 26 with other diseases (Spearman's rank correlation, $R = 0.40$, $2\alpha < 0.01$).

Cl_{ph} in the forenoon could separate patients with PHP from other patients, since patients with PHP did not demonstrate a diurnal variation of Cl_{ph}.

Several investigators (3, 15) have found lower TRP in patients with PHP than in normal persons. Cl_{cr}, however, has also been found to be lower in patients with PHP than in normal individuals. This could explain why TRP is lower in patients with PHP, since Friis (2) has shown that TRP is dependent upon Cl_{cr} when the latter is low. This has been corroborated by the present investigation. Thomas et al. (13) found in patients with PHP and in patients with hypercalcemia of other etiology a lower TRP than in normal individuals. They considered a reduced renal function in the former patients to be the cause of this phenomenon. Strott and Nugent (12) found on analysis of results from several studies that TRP could better differentiate patients with PHP from normal individuals than could Cl_{ph}. They found, however, low TRP values not only in patients with PHP, but also in patients with hypercalcemia on the basis of a malignant disease. In the present study a low TRP was also found in several patients with non-parathyroid hypercalcemia. This could be explained by the fact that these patients had a reduced renal function. The low TRP found in patients with reduced renal function can be explained on the basis of secondary hyperparathyroidism (2). In patients with non-parathyroid hypercalcemia an endogenic suppression of the parathyroid glands could be expected, so that a secondary hyperparathyroidism cannot explain the low TRP in all cases.

TRCa was higher in patients with nephrolithiasis and in the group of patients with other diseases than in patients with PHP. This could be explained on the basis of an increased filtration of the ultrafiltrable calcium fraction in patients with PHP. Poulos (9) and Kleeman et al. (4) have shown that this can cause a reduction in the percental reabsorption of calcium in spite of the fact that the total amount of calcium reabsorbed in the tubuli is increased. The relationship found in the present study between TRCa and the serum calcium concentration in patients with PHP (Fig. 6) could be explained in the same way. The reduced TRCa, which Smith and MacKenzie (11) found in patients with nephrolithiasis in relation to normal persons, could not be reproduced in the present study. This is in agreement with the observations of Peacock and Nordin (8).

No difference was found between TRCa values in patients with PHP and in patients with hypercalcemia of other cause. Very low TRCa values were, however, only found in patients with non-parathyroid hypercalcemia. If the TRCa is correlated to the serum calcium values, it is possible to draw a line parallel with the line of regression, so that in the case of serum calcium values greater than 2.75 mmol/l a complete separation between the groups of patients with PHP and with non-parathyroid hypercalcemia is achieved. Transbøl et al. (14) correlated TRCa to the concentration of ultrafiltrable serum calcium and found a corresponding divergence between 16 patients with PHP and 11 with non-parathyroid hypercalcemia. They found, however, high TRCa values in 2 patients with hypercalcemia, metabolic alkalosis and hypokalemia even though the hypercalcemia was of non-parathyroid origin.

In patients without hypercalcemia the TRCa was much less dependent upon Cl_{cr} than was the TRP. This is in agreement with the observations of Laake (6). In patients with PHP the TRCa was slightly reduced with decreasing renal function, whereas this was more pronounced in patients with non-parathyroid hypercalcemia. A contributing factor may have been an endogenous suppression of the parathyroid glands such as is seen in non-parathyroid hypercalcemia, so that a secondary hyperparathyroidism does not develop with decreasing renal function. Thus, it holds (Fig. 6) that for any serum calcium concentration, a higher parathyroid hormone activity will be accompanied by higher TRCa (4). It could therefore be of differential diagnostic value to determine TRCa in patients with marked hypercalcemia if at the same time the TRCa is correlated to the serum calcium concentration. On the other hand, determination of the TRCa has no value in the differential diagnosis of patients with nephrolithiasis and PHP in cases when the serum calcium concentration is either slightly increased or normal.

Since TRP varies with renal function, measurement of this parameter cannot be used in the diagnoses of PHP in patients with reduced renal function, i.e. $Cl_{cr} < 70$ ml/min. In patients with normal renal function, however, measurement of the TRP may perhaps be of [illegible] value.

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DIURNAL PATTERN OF PLASMA 11 HYDROXYCORTICOSTEROIDS IN ACUTE MYOCARDIAL INFARCTION

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Abstract. Plasma levels of 11-hydroxycorticosteroids (11 OH-CS) over 24-hour period after admission were studied in 19 patients with acute myocardial infarction, divided into two groups. Controls for this study consisted of 10 ambulatory patients without any endocrine diseases. The normal diurnal variation in plasma 11-OH-CS was lacking in most of the patients—the night levels were nearly the same as the morning levels and were higher than normal. The possible mechanisms causing the abolition of variation in plasma 11-OH-CS concentration are discussed. The present findings are of importance when discussing the adrenocortical stimulation during the early phase of myocardial infarction, and might be related to the absence of diurnal rhythm of water and electrolyte excretion in heart failure.

In healthy subjects a diurnal variation in adrenal output of cortisol has been well established. The concentrations of plasma cortisol and ACTH as well are highest early in the morning and decline during the day reaching their lowest levels in the evening (12). The hypothalamic control centre works to a 24-hour rhythm (15). Regardless of the cause of cortisol excess, most patients with Cushing's syndrome fail to show a normal rhythm in plasma cortisol (3 4). In patients with myocardial infarction there is an initial phase in which plasma cortisol concentrations are raised above the normal level (10 13). The diurnal variation in plasma cortisol in this condition has not been studied, and would be of importance for a better understanding of the adrenocortical function in such patients.

In this paper we present the results of plasma 11-hydroxycorticosteroids (11-OH-CS) studied over a 24-hour period in patients in the phase immediately following the clinical onset of myocardial infarction.

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MATERIAL AND METHODS

The diurnal rhythm of plasma 11-OH-CS was studied in three groups:

Group I—controls. This group comprised 10 subjects. They were patients without endocrine disorders or any other diseases that might affect the adrenal function. All of them had been in hospital for several days before the investigation took place.

Group II Nine patients with acute myocardial infarction (AMI). These patients had history of early morning waking with the typical ischaemic pain. They were admitted to hospital between 08.00 hours and noon, with symptoms of less than 6 hours duration.

Group III Ten patients with AMI. These patients developed typical ischaemic chest pain during the daytime and were admitted between 14.00 and 20.00 hours, less than 6 hours after the onset of the disease.

All patients were observed in the Coronary Care Unit, here they were confined to bed.

For the controls venous blood samples were taken at 12-hour intervals at 08.00 and 20.00 hours only. For the patient groups venous blood samples were taken on admission. In group I additional samples were taken at 16.00 and 23.00 hours on the day of admission, and at 08.00 hours on the next day. In group II additional samples on the day of admission were taken at 23.00 hours, and at 08.00 and 16.00 hours on the next day.

Plasma 11-OH-CS levels were determined by the modification of the method of Mattingly (14), described by *De Moor & Steeno (2).*

RESULTS

The results in the three groups are summarized in Fig. 1

Group I—controls. There was a well-marked diurnal rhythm. The values at 08.00 hours were on an average twice the values found at 20.00 hours. These results are similar to those found in healthy subjects by previous workers.

Group II and group III On admission plasma levels of 11-OH-CS were similar in the two

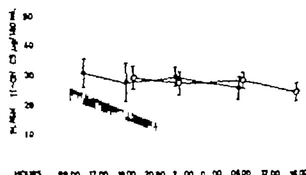


Fig 1 Mean concentration ±1 S.E. of 11-OH-CS in patients with AMI. ● = 9 patients admitted before noon. ○ = 10 patients admitted after noon. The shaded area represents ±1 S.E. from the mean values of 10 control subjects.

groups: mean 31.0 μg/100 ml and 28.9 μg/100 ml for groups II and III, respectively and higher than that of the controls. The mean level and most of the individual levels in the patient groups were maintained during the 24-hour observation period and remained above normal. No significant variation was observed in the mean values during the day

DISCUSSION

In healthy subjects there is approximately a twofold difference between the peak plasma values for 11-OH-CS early in the morning hours and the low values late at night. In the series of experi- presented here, a loss of the normal rhythm of plasma 11-OH-CS has been a typical finding in most patients with AMI.

Previous workers have shown an absence of diurnal rhythm in valvular heart disease throughout an episode of congestive cardiac failure (1, 11, 16). Their results showed a pattern similar to that observed in our patients, although an elevated plasma cortisol level was not always observed by the authors referred to. The disappearance of the normal diurnal rhythm in congestive heart failure is not fully understood. It has been suggested that the circulatory failure leading to reduced hepatic blood flow with subsequently reduced hepatic removal of cortisol from the plasma, may be of primary importance in the disappearance of the diurnal variation (5, 6). This effect could not be a consequence of the stressful situation, since Estep et al. (7) were unable to demonstrate impaired cortisol metabolism during surgical stress. Although this hypothesis cannot be totally dismissed, it does not appear very likely. With an intact and adequately functioning pituitary-adrenal axis, a reduction in metabolic removal of cortisol from the blood would immediately lead to reduced adrenal cortisol output.

Our findings probably reflect increased cortisol production as a response to a prolonged period of stress, combined with changed metabolism due to the circulatory failure. The rise in plasma cortisol in the AMI may be regarded as an emergency reaction. These observations are thus in agreement with the concept presented by Forsham (8) that the central nervous reactions involved in the stress situation override the normal cyclic function of the hypothalamus and the pituitary with regard to ACTH release. It is unlikely that the disappearance of the normal diurnal rhythm is related to confinement to bed, as it was shown by Perkoff et al. (17) that patients who had been bedridden for longer or shorter periods still had a normal diurnal plasma cortisol variation.

The lack of the normal variation in plasma 11-OH-CS levels in most of the patients discussed here is of importance when discussing the adrenocortical response to the stress of myocardial infarction. The observations presented might be related to the known abolition of the diurnal rhythm of water and electrolyte excretion in congestive heart failure (9).

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CONGRESS ANNOUNCEMENT

The First Joint Meeting of International Societies for Hygiene Preventive- and Social Medicine will be held—probably in the Hofburg—in Vienna, Austria, October 29 – November 1 1972.

Inquiries: The Secretary of the Congress, Mrs. E. Weidenhaus, Wiener Medizinische Akademie Stadiongasse 6–8, A 1010 Vienna, Austria.

An International Workshop on Mutagenicity Testing of Drugs and Other Chemicals will be conducted at the Institute of Pathological Anatomy University of Zurich, Switzerland, Oct. 2–5, 1972.

Requests for the program application forms and further information. Prof G Zbinden, Institute of Pathological Anatomy University of Zurich, Kantonsspital, CH-8006 Zurich, Switzerland. (The number of participants will be limited. Applications must be received by Aug. 15 1972.)